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ANNALS OF BOTANY

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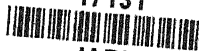ASSISTED BY

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Studies in Tropical Fruits

XIV. Carbohydrate Metabolism of the Banana Fruit during Storage at 53° F.

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With nine Figures in the Text

I. INTRODUCTION

THE overseas transport of bananas is based upon the use of a storage duration and temperature such that the ripening trend of the relatively immature green fruit is slowed down sufficiently for the fruit to be still green and firm at the end of the voyage, and on removal to a higher temperature to ripen to an attractive colour of skin and flavour of pulp within a relatively short period. The maximum storage period at the temperature adopted, 53° F., has been found to vary with the stage of development of the bananas

at harvesting. If this period is exceeded the subsequent ripening changes at a temperature of 68° F. are abnormal and produce a ripe fruit of poor colour and flavour. In a previous contribution to this series (Barnell, 1941a) the carbohydrate changes were followed during storage at 53° F. of two commercial grades of bananas for periods normal for the grade and also when storage was extended sufficiently to produce slight chilling. The subsequent carbohydrate changes during ripening at 68° F. were also determined. In the present paper the effect of continuing storage at 53° F. of a single grade of banana has been followed in order to obtain further information on the metabolism of fruit subjected to extreme chilling.

The estimation of particular selected carbohydrates clearly provides information on the effects of low temperature on restricted aspects of the total metabolic drift in the ripening banana. Simultaneously with the present work other lines of investigation, including studies of banana hemicelluloses and tannins, have been carried out, the data from which are relevant to the present problem; these will be reserved for future communications.

II. MATERIALS AND METHODS

Bunches of comparatively thin, 'heavy ¾-full' Gros Michel bananas (mean weight, on receipt, of a finger from third-hand,[1] upper row, 158·8 gm.; mean pulp/skin weight-ratio, 1·57[1]) were obtained from fruit offered for shipment to Canada. Thirty bunches were selected for uniformity of grade, culled for symptoms of infection with *Cercospora* leaf-spot disease (Wardlaw, 1937), sampled, and placed in cold storage within little more than twenty-four hours from cutting.

The method of sampling and of the preparation of samples for analysis were those previously employed (Barnell, 1941, 1941a); the methods used to extract and estimate the carbohydrates have also been described in earlier papers (Barnell, 1936, 1940). Sampling was restricted to the fingers of the upper rows of the third and fourth hands until near the end of the trial, when due to shortage of material lower-row fingers were taken.

The bunches were arranged around the walls of a storage room so as to be accessible for inspection and sampling. The air temperature of the room was maintained at 53° F. by the battery system of refrigeration and the relative humidity fluctuated between 80 and 85 per cent. This relative humidity is rather lower than that usually attained in commercial practice and is lower than those used by Leonard and Wardlaw (1941), namely, 85 and 100 per cent.

III. BEHAVIOUR OF FRUIT DURING STORAGE

The thirty bunches cooled to the air temperature of 53° F. within twelve hours of placing in the storage room.

Table I summarizes the periodic observations which were made on the

[1] For explanation of terms see Wardlaw, Leonard, and Barnell, 1939.

bunches. Within 7 days from cutting 5 out of the 30 bunches showed colouring, i.e. premature ripening, as a result, in these instances, of *Cercospora* infection as indicated by the flesh colour. These bunches were removed on the 7th day, and the trial continued with 25 bunches. These were apparently

TABLE I

Number of Bunches showing Different Ripening Symptoms

Days from cutting.	Firm green.	Colour-ing.	'Yellow.'	Chill colours.	Skin dark. Pulp firm.	Skin dark. Pulp soft.	Skin black. 'Over-ripe.'
7	25	5*	0	0	0	0	0
10	25	0	0	0	0	0	0
20	18	7	0	3	0	0	0
30	4	18	3	19	0	0	0
40	0	7	4	25	14	0	0
46	0	2	3	25	20	0	0
60	0	0	1	25	0	24	0
86	0	0	0	0	0	11	14
99	0	0	0	0	0	0	25

* Five bunches from *Cercospora* infected plants showed premature ripening and were removed after 7 days, leaving 25 bunches for the remainder of the storage trial.

normal in behaviour. The majority of bunches showed the first stage of ripening, colouring, and attaining the 'sprung' condition between 20 and 30 days from the date of cutting. By the 40th day no firm green bunches remained; all showed, according to their stage of ripeness, the sooty yellow or bronze colours produced by chilling. After 40 days the fruit passed slowly through the stages of skin colour: bronze, bronze-brown (pulp firm), brown (pulp soft) to, finally by the 99th day, black with liquid pulp and considerable white superficial fungus mycelium. There was some main-stalk rot, but until a few days before the end of the storage period no fingers were dropped from this cause.

IV. The Pulp/Skin Weight-ratio and Water Relations of the Pulp and Skin

The use of the pulp/skin weight-ratio as an index of ripeness has been discussed elsewhere (Barnell, 1941*a*).

The mean fresh weights of whole fingers, of pulps and skins of single fingers together with the corresponding values of the pulp/skin weight-ratio have been plotted in Fig. 1. The values for the whole finger weight fell fairly steadily over the entire period of storage. The pulp weight fell slowly at first, then after 20 days, rose to a peak value at approximately 60 days, and then fell continuously to the end of the period. The skin weight fell continuously over the whole period but more rapidly between 20 and 40 days than at other times.

The pulp/skin ratio remained approximately constant up till about 20 days, after which it rose steadily, reaching a value of 2·06 on day 37, and eventually

a peak value of 2·90 on day 75; after this falling values were recorded. Two observations can be made from these data: (i) a pulp/skin weight-ratio of 2·0 was found to be associated with the 'eating-ripe' condition in fruit at tropical
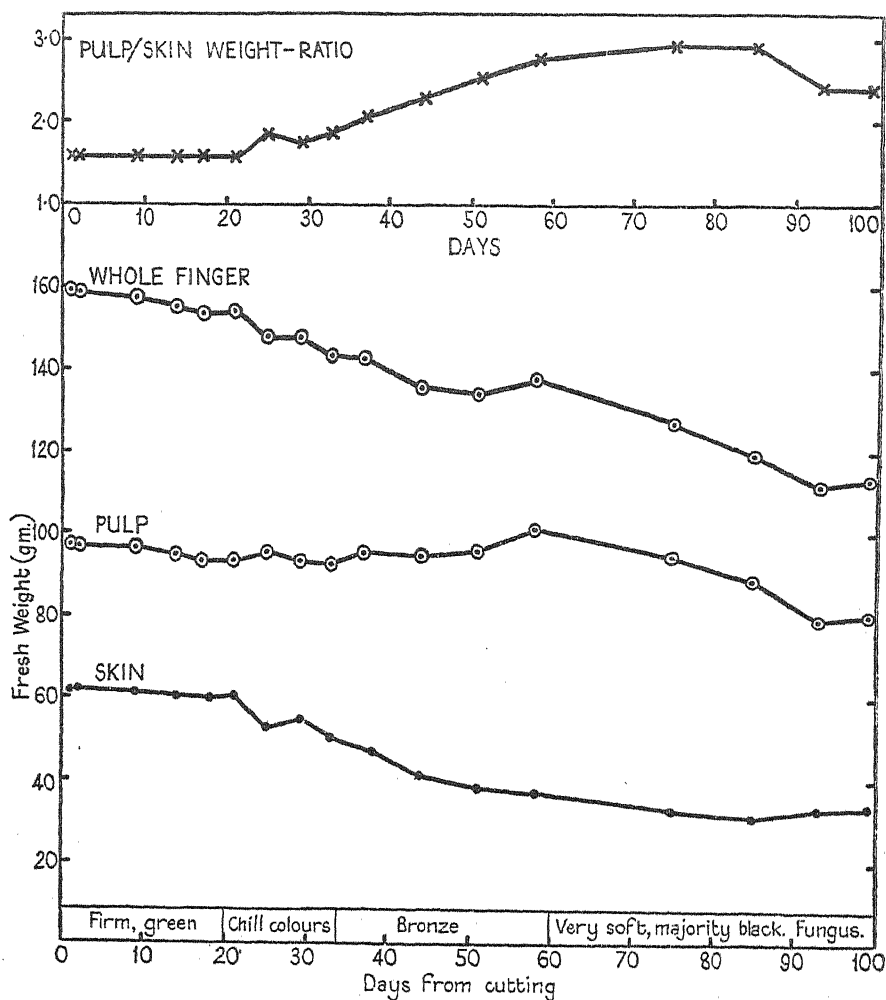
FIG. 1. The mean fresh weights (gm.) of whole fingers, pulps, and skins together with the pulp/skin weight ratios of 'heavy ¾-full' bananas during storage at 53° F. Observations on the appearance of the skin are inserted along the time axis.

temperatures, and at 68° F. after storage at 53° F. During continuous storage at 53° F. this ratio is attained considerably earlier than is the composition of the pulp previously associated with it (data in Fig. 1). (ii) If storage at 53° F. is sufficiently prolonged the ratio shows falling values, though this may be due, to some extent, to the use of fingers from the lower rows of hands in the late stages of the storage trial.

In bananas which had been 'chilled' by a relatively short prolongation of the refrigerated storage period beyond that tolerated by their grade it has been observed (Barnell, 1941*a*) that the loss in weight of the skin, during the period in which the pulp weight was increasing, almost exactly equalled the gain in weight of the pulp. In fruit not subjected to chilling the skin-
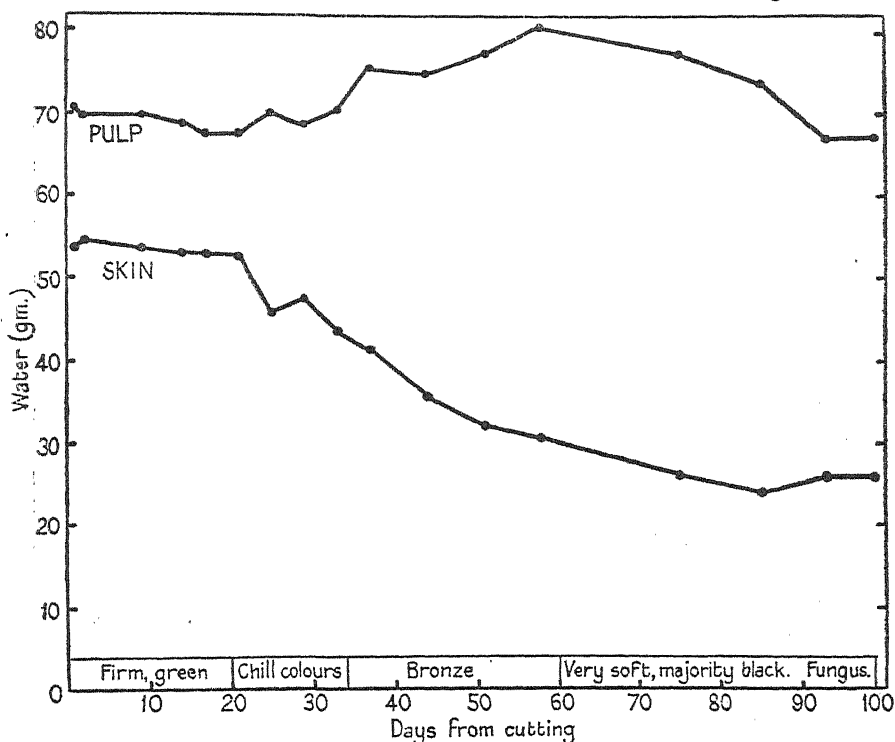
FIG. 2. Total amounts of water (gm.) per single pulp and per single skin of 'heavy ¾-full' bananas during storage at 53° F.

weight loss was considerably in excess of the pulp-weight gain. It was suggested that chilled fruits had reduced transpiration rates or that the transpiration loss might be counterbalanced by intake of water by the finger from the bunch stalk or both.

The data for the water relations of pulp and skin in the present investigation have been plotted in Fig. 2 in the form of mean water contents per single pulp and per single skin. This method gives a clearer presentation of the water interrelations of pulp and skin than is obtained from the mean fresh weights of pulp and skin.

A consideration of Fig. 2 shows that the main phase of water-uptake by the pulp was between 20 and 60 days and that this phase coincided with that of the main water loss by the skin. The amount of water lost by the skin over this period was approximately twice that taken up by the pulp. This agrees, on an extended time basis, with the effect noted during ripening at 68° F. of

fruit not previously subjected to chilling; the suggestion is therefore made that the balanced relation of water loss by the skin and water uptake by the pulp is only to be observed in chilled fruit ripening at a temperature above that producing chill effects, i.e. it is a residual effect of changes induced in the skin by moderate chilling.
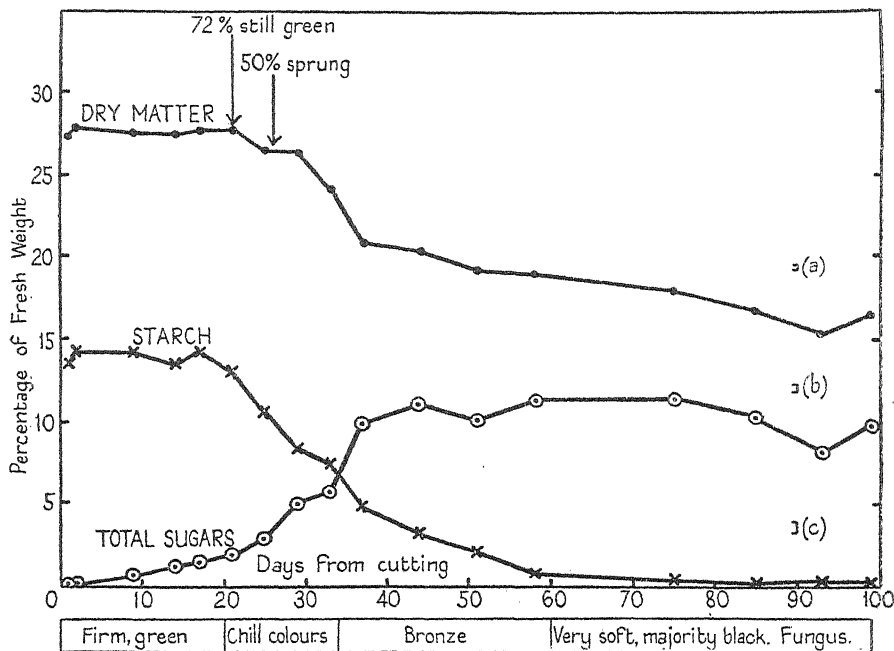
FIG. 3. *Pulp.* Dry matter, starch, and total sugars as percentages of the fresh weight during storage at 53° F. The vertical lines labelled (*a*), (*b*), and (*c*) represent the minimum significant differences (*P* = 0·05) of (*a*) dry matter, (*b*) total sugars, and (*c*) starch.

The prolonged and slow rise in the water content of the pulp at 53° F. is associated with the slow starch hydrolysis and the rates of sugar accumulation discussed below.

V. PERCENTAGE AMOUNTS OF DRY MATTER AND VARIOUS CARBOHYDRATES IN THE PULP

Figs. 3 and 4 represent graphically the data for dry matter and the various estimated carbohydrates, expressed as percentages of the fresh weight of pulp. The associated colour changes of the skins of the fruits are indicated along the time axes.

(*a*) *Total dry matter, starch, and total sugars.*

These are plotted in Fig. 3. There was little change in the dry matter percentage over the first 20 days (cf. Barnell, 1941*a*, Fig. 5); after 20 days the percentage fell, the rate of fall increasing with time up to approximately

40 days, when a relatively slow steady fall occurred. Starch also showed little change during the first 20 days (cf. Barnell, 1941*a*, Fig. 5, where an increase for 9 days followed by a decrease was observed), but afterwards decreased in the manner previously observed in the pulps of fruits ripening

TABLE II

Rate of Loss of Starch of Pulp

(1) Grade.	(2) Days at 53° F.	(3) Ripening temperature.	(4) K (index of starch-loss rate).
'Heavy ¾-full'	0	Room (80–85° F.)	0·250*
,,	7	68° F.	0·180*
,,	14	68° F.	0·135*
,,	99	53° F.	0·028

* Data from previously recorded work; Barnell (1941), (1941*a*, Table VII).

at tropical temperatures and at 68° F. However, at 53° F. approximately 40 days, instead of the 7 or 8 at tropical temperatures, were required for the starch percentage to attain the low values to be found normally in 'eating-ripe' fruit.

The sensitivity of the starch-hydrolysis complex to the temperature of storage is shown in Table II by a comparison of the values of the index of starch-loss rate, K (cf. Barnell, 1941*a*, p. 639), for the present fruit ripening at 53° F. with those for fruit ripening at tropical temperatures or at 68° F., after various storage periods at 53° F. The fruit stored throughout at 53° F. has a very low index (cf. Barnell, 1941*a*, Table VII).

The total sugar percentage rose simultaneously with the starch fall, but the values of the sustained level of total sugar from 40 to 80 days did not reach the initial starch level. Slightly falling values were observed for the total sugars after 80 days.

(b) Sucrose, glucose, fructose, and glycosidic-glucose.

The sucrose percentage rose steadily to a peak value on the 43rd day (Fig. 4). In previous records of fruit ripening at higher temperatures this peak value coincided with the 'eating-ripe' stage; at 53° F. the skin had developed well-marked bronze colours, whilst the pulp remained fairly firm though softening from the centre outwards. Falling values of sucrose percentage were recorded during the remainder of the storage period except for the final estimation. Glucose and fructose were present in approximately equal quantities during the first 10 to 15 days. After 10 days both sugars increased in percentage amount, glucose slowly at first but later with increasing speed until by the 37th day their proportions were again approximately those of invert sugar; after that day some divergence occurred, but both sugars attained their peak value on the 73rd day. Fructose was significantly higher than glucose in the last two observations.

There was no evidence of appreciable glycosidic-glucose formation until after 24 days when an increase occurred, continuing to a peak value on the 50th day. The values attained during the period corresponding to the 'eating-
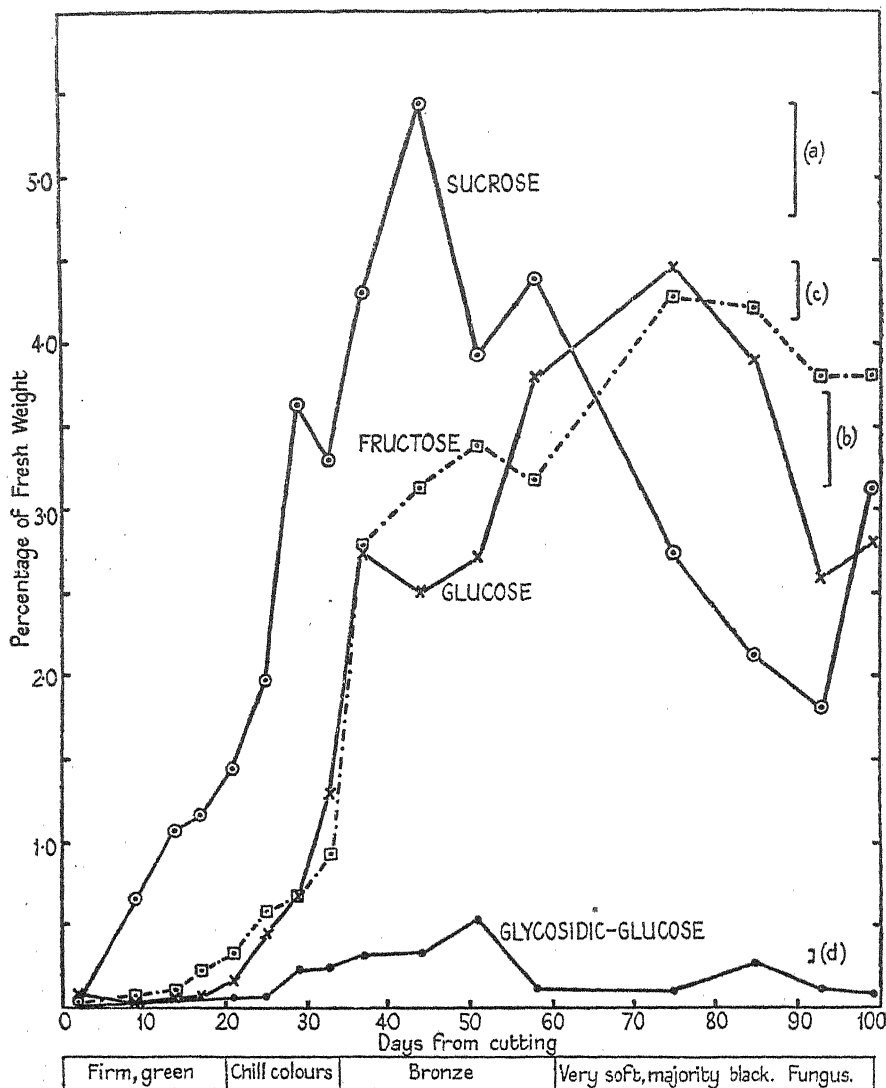
FIG. 4. *Pulp.* Sucrose, glucose, fructose, and glycosidic-glucose as percentages of the fresh weight during storage at 53° F. The vertical lines labelled (*a*), (*b*), (*c*), and (*d*) represent the minimum significant differences of (*a*) sucrose, (*b*) glucose, (*c*) fructose, and (*d*) glycosidic-glucose.

ripe' stage, from the 45th to the 60th day approximately, were not as high as might have been anticipated from previous data on the effects of 'chilling' on the glycosidic-glucose content of the pulp (Barnell, 1941*a*).

With these exceptions the trends of the various sugars in the pulp during ripening were, on an extended time basis, similar to those observed in bananas ripening at higher temperatures.
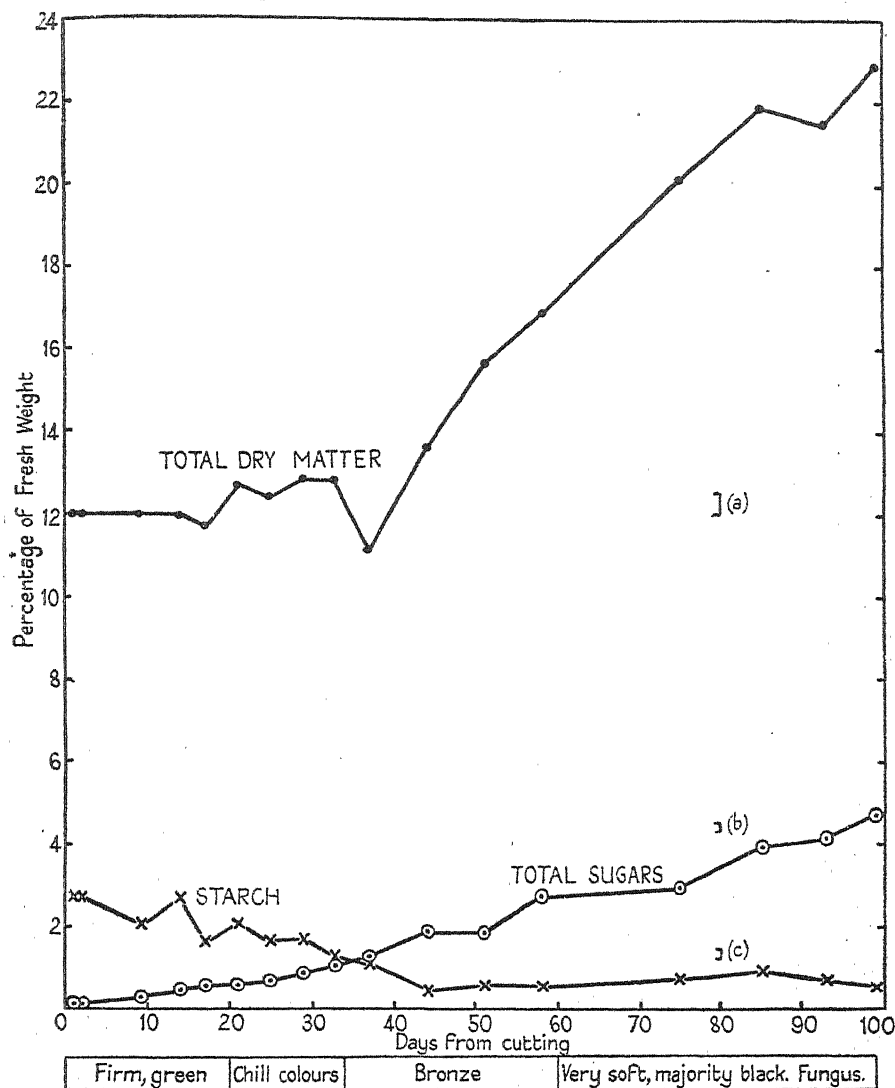
FIG. 5. *Skin.* Dry matter, starch, and total sugars as percentages of the fresh weight during storage at 53° F. The vertical lines labelled (*a*), (*b*), and (*c*) represent the minimum significant differences of (*a*) dry matter, (*b*) total sugars, and (*c*) starch.

VI. PERCENTAGE AMOUNTS OF DRY MATTER AND VARIOUS CARBOHYDRATES IN THE SKIN

The data for the dry matter and carbohydrate, as percentage of fresh weight, in the skin are set out in Figs. 5 and 6.

(a) Total dry matter, starch, and total sugars.

These are plotted in Fig. 5. The dry matter percentage showed little change during the first 20 days. Apart from one low value it then rose consistently, slowly at first but rapidly after 40 days. This increasing percentage of dry matter in the skin was entirely due to water loss and not to any actual
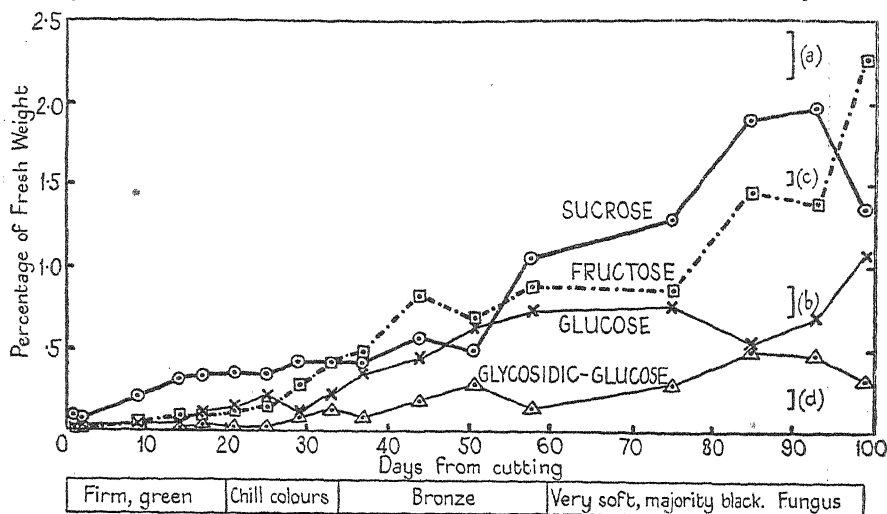
Fig. 6. *Skin.* Sucrose, glucose, fructose, and glycosidic-glucose as percentages of the fresh weight during storage at 53° F. The vertical lines labelled (a), (b), (c), and (d) represent the minimum significant differences of (a) sucrose, (b) glucose, (c) fructose, and (d) glycosidic-glucose.

increase in dry matter present (see section IX (*b*)). The starch percentage fell with some slight irregularities until the 44th day, after which practically constant values were recorded. The declining water content was largely responsible for maintaining the starch percentage at this level during this final period. Total sugars increased in percentage amount steadily and continuously over the entire period.

(b) Sucrose, glucose, fructose, and glycosidic-glucose.

Sucrose increased from the first observation with a relatively level stage between 15 and 50 days, after which the rise continued more steeply, falling, however, to the last value (Fig. 6). It was the sugar present in highest percentage over the greater part of the period. Glucose and fructose were approximately equal in percentage amounts during the first 25 days, but afterwards fructose increased to a considerably greater extent than glucose. Glycosidic-glucose presented a somewhat variable drift with apparent peak values at 51 and 85 days.

VII. Titratable Acid in Pulp and Skin

Data for the titratable acid in pulps and skins are set out in Table III; in (*a*) as ml. N/10 NaOH per 100 gm. fresh weight; in (*b*) as ml. N/10 NaOH

per single pulp or per single skin. These acid estimations, for reasons previously discussed (Barnell, 1941a), are of a relatively low order of accuracy and further investigations are necessary before the changes in acid content are established.

Allowing for the variability of the data there is little change in the 'percentage' of acid in the pulp (column 3, Table III) during the first 30 days.

TABLE III

Titratable Acid in Pulp and Skin

	Days from cutting.	(a) ml. N/10.NaOH per 100 gm.		(b) ml. N/10.NaOH per single pulp or skin.	
		Pulp.	Skin.	Pulp.	Skin.
1	1	34·7	17·4	33·7	10·7
2	2	—	23·7	—	14·6
3	9	37·4	19·8	36·0	12·0
4	14	39·3	24·7	37·2	14·8
5	17	40·3	23·2	37·6	13·9
6	21	36·2	23·2	33·9	14·0
7	25	—	19·2	—	10·0
8	29	39·0	29·4	36·4	16·0
9	33	34·1	23·3	31·6	11·7
10	37	50·9	27·4	48·7	12·8
11	44	50·9	39·1	48·0	16·0
12	51	55·8	35·1	53·4	13·8
13	58	47·3	40·2	47·7	14·8
14	75	44·3	45·3	41·7	14·7
15	85	44·3	40·3	39·3	12·4
16	93	47·1	45·4	37·2	14·9
17	99	38·3	42·7	30·0	14·3
Significant difference (P = 0·05):		9·6	5·1	—	—

After this values rise to a peak some time between 40 and 50 days; the highest value observed was on day 50. Falling values were afterwards recorded to the end of the period. The peak value region corresponds with the beginning of the 'eating-ripe' stage as indicated by the starch and sugar values; this is similar to the behaviour of acid in fruit ripened at higher temperatures. An essentially similar drift is given by the data for the pulp when expressed on a basis of 'total amount per pulp' (column 5, Table III).

The general drift of the 'percentage' acid in the skin (column 4 in Table III), again allowing for variability, rose slowly at first, then relatively quickly between 30 and 50 days attaining a maximal value at approximately 70 days which was maintained to the end of the period. The same data for the skin expressed as ml. N/10 NaOH per skin (column 6 in Table III) present a very different trend; there is no evidence of any changes beyond variation about a mean value over the whole 99-day period, i.e. the loss of water from the skin considerably modifies the expression of the data.

VIII. PERCENTAGE AMOUNTS OF TOTAL ALCOHOL-SOLUBLE
SUBSTANCES IN PULP AND SKIN

The amounts of total alcohol-soluble substances (sucrose+glucose+fructose+'non-sugars') as percentages of the fresh weight are set out in Table IV for extension of and comparison with similar data given in previous papers of this series.

Apart from the extended time basis, common to all the substances estimated, the changes observed in the various fractions are similar to those in

TABLE IV

Total Alcohol-soluble Substances (total sugars and 'non-sugars') in Pulp and Skin (as percentages of the fresh weight)

	Days from cutting.	Pulp.			Skin.		
		Total alcohol-soluble substances.	Total sugars.	Non-sugar (by difference).	Total alcohol-soluble substances.	Total sugars.	Non-sugar (by difference).
1	1	1·01	0·09	0·92	1·15	0·11	1·04
2	2	1·49	0·13	1·36	1·43	0·12	1·31
3	9	2·15	0·75	1·40	1·81	0·30	1·51
4	14	2·58	1·24	1·34	2·04	0·44	1·60
5	17	2·95	1·48	1·47	1·81	0·55	1·26
6	21	3·53	1·94	1·59	2·03	0·59	1·44
7	25	5·18	2·94	2·24	2·56	0·68	1·88
8	29	8·25	4·97	3·28	3·13	0·85	2·28
9	33	8·54	5·51	3·03	3·63	1·05	2·58
10	37	12·85	9·90	2·95	3·96	1·28	2·68
11	44	14·66	11·07	3·59	4·44	1·83	2·61
12	51	14·52	10·03	4·49	4·77	1·80	2·97
13	58	15·17	11·37	3·80	5·21	2·71	2·50
14	75	14·61	11·48	3·13	6·51	2·90	3·61
15	85	13·91	10·24	3·67	7·96	3·88	4·08
16	93	12·39	8·19	4·20	8·24	4·05	4·19
17	99	13·08	9·76	4·32	7·93	4·70	3·23

bananas ripening at higher temperatures (Barnell, 1941). In both pulp and skin of the freshly harvested, green banana the total alcohol-soluble substances consisted very largely of the 'non-sugar' fraction. The proportion of this fraction to total sugars decreased with time and did so more rapidly in the pulp than in the skin.

The drifts of the alcohol-soluble non-sugar fraction in the pulp and in the skin were similar to those of the total sugars in that they increased as 'ripening' proceeded. It may therefore be restated that—considering the magnitude of the non-sugar fraction and of the changes undergone by it during development and 'ripening'—this fraction must be considered to contain one or more substances of importance as metabolites (Barnell, 1941, 1941a).

Apart from some preliminary acid, glycosidic-glucose, and tannin deter-

minations it has not yet been possible to examine more closely the constituents of this fraction.

IX. CHANGES IN THE TOTAL AMOUNTS OF DRY MATTER AND VARIOUS CARBOHYDRATES IN THE PULP AND SKIN

The method of presenting the data for the composition of the banana fruit in terms of absolute amounts per single pulp and per single skin has been

TABLE V

Pulp Weight (gm.) and Amounts (gm.) of various Carbohydrates in Pulp of single Finger

Days from cutting.	Pulp wt.	Total dry matter.	Glucose.	Fructose.	Sucrose.	Total sugars.	Starch.	Glycosidic glucose.	Total estimated carbohydrates.
1	97·1	26·37	0·046	0·006	0·038	0·090	13·05	0·010	13·150
2	96·6	26·98	0·091	0·000	0·033	0·124	13·72	0·019	13·863
9	96·4	26·50	0·022	0·068	0·632	0·722	13·61	—	14·332
14	94·7	25·96	0·049	0·112	1·018	1·179	12·72	—	13·899
17	93·3	25·01	0·071	0·219	1·091	1·381	13·22	—	14·601
21	93·6	26·05	0·152	0·306	1·351	1·809	12·11	0·053	13·972
25	95·2	25·21	0·425	0·491	1·881	2·797	10·02	0·061	12·878
29	93·4	24·58	0·625	0·625	3·392	4·642	7·83	0·212	12·704
33	92·7	22·37	1·192	0·861	3·046	5·099	6·82	0·222	12·141
37	95·6	19·91	2·628	2·722	4·120	9·470	4·67	0·342	14·482
44	94·2	19·16	2·361	2·940	5·131	10·432	2·95	0·351	13·733
51	95·8	18·36	2·607	3·254	3·761	9·622	2·01	0·515	12·147
58	100·9	19·11	3·216	3·826	4·431	11·473	0·70	0·113	12·286
75	94·1	16·72	4·196	4·024	2·568	10·788	0·38	0·103	11·270
85	88·7	14·81	3·457	3·742	1·887	9·086	0·14	0·238	9·459
93	79·1	12·08	2·042	3·007	1·430	6·479	0·28	0·080	6·839
99	80·4	13·26	2·259	3·073	2·518	7·850	0·30	0·064	8·216

adopted in previous papers of this series, where its advantages have been discussed. Provided sampling variation is not too great this method allows actual gains or losses of individual constituents in the two readily separable components of the banana fruit to be followed over a period.

The present data expressed on this basis are given for the pulp in Table V and for the skin in Table VI.

(a) Pulp

Figures showing changes in the amounts of the individual constituents of the pulp have not been presented since the forms of the curves constructed from the data given in Table V differ but slightly from the percentage data already illustrated (Figs. 3 and 4). The chief reason for this similarity lies in the relatively small changes at 53° F. in the amount of water present in the pulp (Figs. 1 and 2).

(b) Skin

The data for total dry matter, total sugars, and starch from columns 3, 7, and 8 of Table VI have been plotted in Fig. 7. The absolute amount of
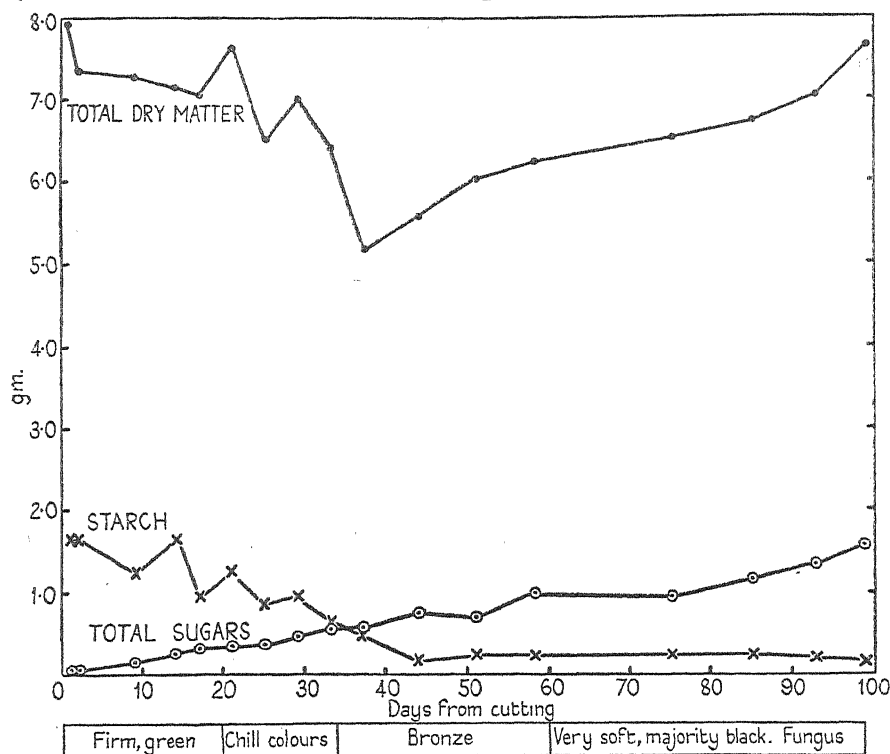
FIG. 7. *Skin.* Amounts (gm.) of dry matter, starch, and of total sugars in skins of single fingers at various stages of ripening during storage at 53° F.

TABLE VI

Skin weight (gm.) and Amounts (gm.) of various Carbohydrates in Skin of single Finger

Days from cutting.	Pulp wt.	Total dry matter.	Glucose.	Fructose.	Sucrose.	Total sugars.	Starch.	Glycosidic glucose.	Total estimated carbohydrates.
1	61·6	7·93	0·009	0·002	0·058	0·069	1·651	0·012	1·732
2	61·7	7·36	0·019	0·011	0·043	0·073	1·647	0·007	1·727
9	60·8	7·29	0·027	0·032	0·122	0·181	1·242	0·010	1·433
14	60·1	7·17	0·029	0·055	0·183	0·267	1·618	0·012	1·897
17	59·9	7·02	0·070	0·047	0·201	0·318	0·961	0·023	1·302
21	60·3	7·67	0·080	0·067	0·205	0·352	1·249	0·016	1·617
25	52·3	6·51	0·106	0·075	0·177	0·358	0·848	0·017	1·223
29	54·4	7·00	0·060	0·178	0·226	0·464	0·934	0·051	1·449
33	50·1	6·41	0·112	0·204	0·210	0·526	0·621	0·065	1·212
37	46·6	5·18	0·161	0·223	0·189	0·573	0·498	0·039	1·110
44	40·9	5·57	0·184	0·337	0·228	0·749	0·167	0·076	0·992
51	38·2	6·01	0·240	0·263	0·185	0·688	0·222	0·108	1·018
58	36·9	6·24	0·285	0·324	0·390	0·999	0·205	0·054	1·258
75	32·5	6·51	0·244	0·278	0·421	0·943	0·232	0·094	1·269
85	30·7	6·72	0·155	0·446	0·582	1·183	0·258	0·150	1·591
93	32·8	7·04	0·224	0·457	0·645	1·326	0·204	0·151	1·681
99	33·4	7·66	0·363	0·753	0·452	1·568	0·166	0·101	1·835

total dry matter fell from the beginning of the storage period until day 37, afterwards rising gradually but steadily until the end. The early part of this curve contrasts with that in Fig. 5 in which the dry matter percentage remained approximately constant for the first 20 days, the falling water content (Fig. 2) masking the actual loss of dry matter that occurred. After 37 days the absolute amount of dry matter in the skin clearly increased since a rise occurs in both Figs. 5 and 7. This accretion of material must have come either (*a*) from the pulp or (*b*) from the bunch stem. From a general knowledge of the intimate relation between pulp and skin, particularly in relation to water movement, it seems that (*a*) is more probable than (*b*). The pulp (Table V, column 3) lost over this period (37 to 99 days) more than sufficient dry matter to cover the increase observed in the skin. At the same time it should be noted that the vascular connexion between skin and stem is better than that between skin and pulp.

The nature of the substance or substances migrating requires some examination. In Fig. 7 it will be seen that up till approximately 44 days sugar accumulation was equalled or exceeded by starch loss, but after that date the starch remained at an approximately constant value while sugars continued to accumulate. The accumulation of total sugars after the 44th day, however, accounts for rather less than half of the total dry matter increase. From data of hemicellulose and cellulose content of the pulps and skins of this same population of bananas, to be submitted in a later paper, it may be mentioned that there is evidence that the remainder of the dry matter accumulation in the skin consisted of hemicelluloses.

The data for the remainder of the estimated carbohydrates in the skin (sucrose, glucose, fructose, and glycosidic-glucose) have not been plotted, but are given in the appropriate columns of Table V.

X. Utilization in Respiration of Dry Matter and Estimated Carbohydrates

In Fig. 8 are plotted the total amounts per finger of dry matter for pulp+skin (data from columns 3 in Tables V and VI). From the smoothed curve drawn through the plotted points the rates of loss of dry matter per week for the whole finger have been calculated and the stepped graph of Fig. 9 constructed.

This stepped graph represents the respiration rate of a single finger assuming that loss of dry matter in respiration resulted from the breakdown of hexoses to carbon dioxide. The form of the curve in Fig. 9 is in general accord with the data presented for the respiration of a single detached finger at 53° F. and 100 per cent. relative humidity (Leonard and Wardlaw, 1941). The rate of dry matter loss was constant until approximately 20 days, after which a rise occurred until a peak value was attained about 30 days after cutting, followed by a fall. After the fall an approximately constant rate occurred between 40 and 70 days. The rising values observed after 70 days

were, most probably, due to the respiratory activity of the fungus mycelium which was increasingly covering the blackening skin of the fruit during the last 30 days. The banana finger together with the fungal infections at this time may be regarded as a unit; the fruit was at a low level of metabolic
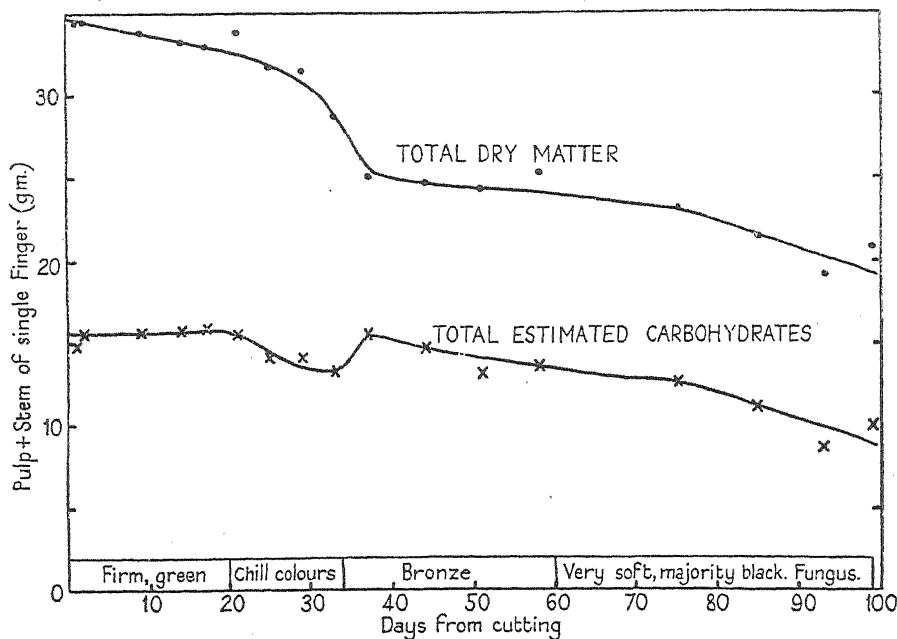
FIG. 8. *Whole finger.* The combined amounts for pulp and skin plotted for total dry matter as dots and for total estimated carbohydrates as crosses. Smooth curves are drawn through the points.

activity, but the fungi were relatively very active, and the material for both mycelial growth and respiration was provided by the fruit with consequent increasing rates of loss of dry matter with increasing severity of fungal exploitation.

A very considerable discrepancy is evident, however, between the rates of dry matter loss plotted in Fig. 9 and the rates of carbon dioxide production given for single fingers at 53° F. by Leonard and Wardlaw (1941). Their value for the constant respiration rate between zero and 20 days was approximately 17 mg. CO_2/kg./hr. and at the climacteric peak, 35 mg./kg./hr. (Leonard and Wardlaw, 1941, Table V). The corresponding values from Fig. 9 for dry matter rates of loss, transformed into mg. CO_2 produced per kg. per hour, were 38·5 and 214·1, assuming all dry matter loss was in the form of carbohydrate and that this was completely aerobically respired. Similar discrepancies were found at tropical temperatures (Barnell, 1941).

Leonard and Wardlaw (1941) provide data of the total loss of fresh weight of a single finger over a period under their conditions of detached fingers and 85 per cent. relative humidity; from these it is possible to deduce that

the dry matter loss under their conditions was comparable with that found in the present work. In their Table VII the loss in fresh weight of Finger 2 over 101·5 days was 60·44 gm.; assuming the average value, 19·82 per cent.,[1] for the dry matter content of whole fingers this represents a loss in dry
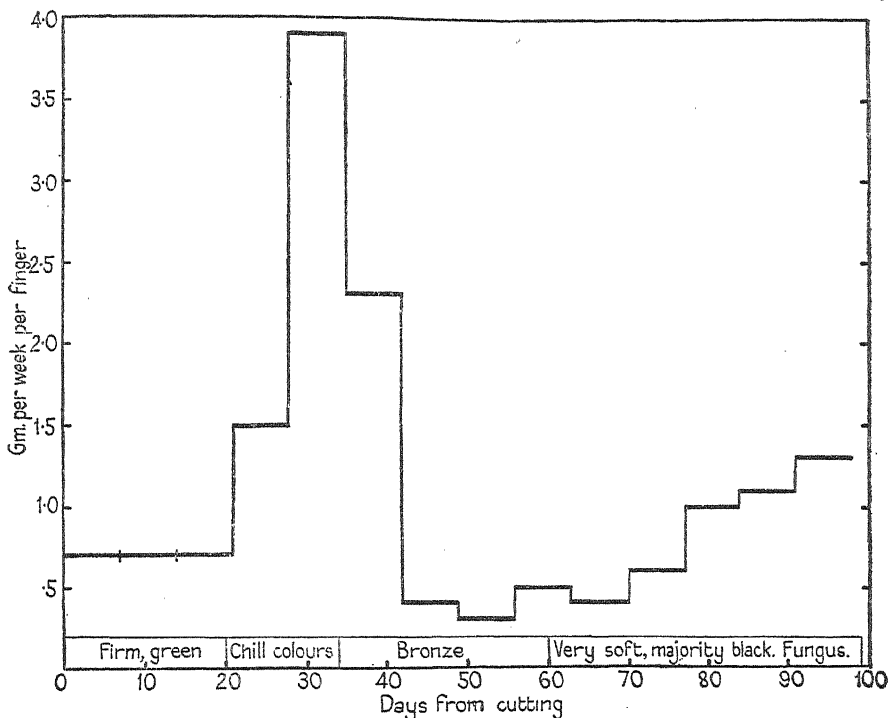
FIG. 9. Weekly rates of loss of total dry matter by the whole finger throughout the storage period at 53° F. The plotted values were obtained by calculation from the smooth curve for amounts of total dry matter shown in Fig. 8.

weight of 12·0 gm. The loss in dry weight per finger observed in the present work over a 99-day period was 13·38 gm. (Tables V and VI, column 3).

The discrepancy between the total dry matter loss and the loss due to carbon dioxide production may be due to fallacies in the assumptions (i) that all dry matter loss was in the form of carbohydrates, and (ii) that respiration was completely aerobic. It may also be due, in part, to inherent errors in the method of determining dry matter. As previously mentioned (Barnell, 1941), the occurrence of anaerobic respiration in varying proportions to the total respiration would produce considerable differences in respiration rates as assessed by the two methods of estimation. Changes in the amounts of relatively volatile substances in the tissues would produce apparent losses in dry matter as determined in the present work. The total dry matter was

[1] This value was obtained by averaging the values found in the present work for the dry matter content of whole fingers at 53° F.

estimated by drying the alcohol-extracted residue and an aliquot of the alcohol extract at 100° C.; it is therefore possible for substances only moderately volatile to be lost both during the alcohol extraction and during the subsequent drying. Since the liberation of volatile substances increases during the ripening of the banana fruit (Gane, 1936) it is probable that as the ripening progresses increasing quantities of more or less volatile substances will be lost during the alcohol extraction and drying processes, giving increasingly exaggerated values for rates of dry matter loss. Esters, alcohols, and volatile acids are possible substances contributing to this loss.

After 33 days the total estimated carbohydrates in the whole finger (Fig. 8) rose to approximately their initial value and then fell from this level, the curve being almost parallel with the one for total dry matter. The relatively sudden increase in the estimated carbohydrates between 33 and 37 days was contributed entirely by the pulp (Table V, column 10). Apparently, at this stage the rate of formation of sugars from reserve carbohydrate substances, e.g. starch and hemicelluloses, exceeded the rate of utilization of sugars in respiration and the formation of volatile substances.

In bananas ripening at tropical temperatures (Barnell, 1941) the dry matter lost between the two peak rates of loss consisted to a small extent only of the total estimated carbohydrates. In the present fruit ripening at 53° F. there was no second peak of dry matter loss (Fig. 9); and for the whole period following the peak rate, i.e. from 37 days on, the entire dry matter loss for the finger can be accounted for by the loss in total estimated carbohydrates.

XI. Discussion

The survey of some aspects of the carbohydrate metabolism of Gros Michel banana fruits stored for varying periods at 53° F. followed by ripening at 68° F. (Barnell, 1941*a*) has now been extended by a study of the changes occurring during storage continuously at 53° F.

(a) General effects of storage at 53° F.

It has been stated (Wardlaw and McGuire, 1931) that badly chilled Gros Michel bananas 'failed to ripen'; Leonard and Wardlaw (1941), however, noted: 'although the disappearance of starch at the lower temperature is greatly retarded, nevertheless fruits held until final senescence at 53° F. eventually show an almost complete disappearance.'

The present work has shown that the carbohydrate changes, although greatly retarded, proceeded in the same general direction and were of the same general type as in fruit ripening at higher temperatures, and that eventually the composition of the pulp with respect to starch and sugars was indistinguishable from that of a fruit accepted as 'eating-ripe'. There was, however, a considerable divergence in the time of occurrence of the starch-sugar changes from the times of occurrence of other processes in the fruit. (i) This is particularly noticeable in respect of skin colour changes; when

the skin had changed from green to dull yellow with bronze colours appearing, the starch percentage of the pulp was still high and the sugars low. By the time the starch and sugar values had attained the 'eating-ripe' levels the skin was blackening and the appearance of the chilled fruit very unprepossessing; fungal mycelium was already appearing. (ii) The pulp/skin weight-ratio attained a value of 2·0 at a considerably earlier stage than that indicated by the starch and sugar levels of the pulp as 'eating-ripe'. This 'coefficient of ripeness' has been shown in previous work (Barnell, 1941a) to be in good accord with carbohydrate composition criteria for fruit ripening at a higher temperature. This difference at 53° F. is due to the relatively high rate of loss of water from the skin and to the slow uptake by the pulp, the latter, in turn, being due to the slow rate of sugar formation in the pulp. The amount of water taken up by the pulp during its period of absorption was considerably less than the amount lost by the skin over the same period, whereas in chilled fruit ripening at 68° F. it has previously been observed that these amounts approximately balance (Barnell, 1941a).

(b) *Some aspects of carbohydrate metabolism in pulp and skin of bananas at 53° F.*

It is clear from the data given in the foregoing sections that storage at 53° F. does not produce any marked deviations in the *type* of carbohydrate katabolic processes of the ripening banana fruit from those at higher temperatures; on the whole it merely slows down the speeds of the various reactions. The deviations observed in ripening bananas at 53° F. are due to the differential effects of temperature on the *rates* of the different processes involved in ripening. It has previously been shown (Barnell, 1941a) that the index of starch loss rate at 68° F. is in inverse relation to the period of storage at 53° F. The very low value obtained for the index in the present work was due to the fact that the starch hydrolysis occurred at the low temperature. During ripening considerable amounts of hemicelluloses disappear, presumably by hydrolysis to sugars and other substances, and soluble astringent tannins are precipitated or oxidized and rendered non-astringent and inactive as diastase inhibitors. Evidence will be produced in a later publication that the effect of temperature on the hemicellulose hydrolysis rate differs markedly from that on the starch hydrolysis rate. When once started, the hemicellulose hydrolysis rate, to all intents and purposes, is independent of the temperature of ripening and of the previous storage history, within the limits so far investigated.

The tannin metabolism of the banana is somewhat complicated and will be only briefly mentioned here; a fuller treatment will be given later. In the pulp of chilled fruit, possibly due to high resistance of the tissue to oxygen diffusion, tannin oxidation does not occur to any appreciable extent and discoloration is not observed; tannin precipitation is slow—if precipitation is in the form of a tannin-carbohydrate complex the slow precipitation may be due to the slow starch hydrolysis rate—so that when the starch and sugar

levels are those of an 'eating-ripe' pulp, astringent tannins remain. In the skin the precipitation of tannins is also delayed but oxidation of tannins to coloured compounds occurs, giving rise to the bronze and brown-black colours characteristic of the skins of badly chilled bananas.

(c) Application of results to future banana storage investigations.

It is now possible to restate the desiderata for future banana storage investigations designed to maintain quality in the ripened fruit with an increased storage period. Such storage treatments must: (1) retard the hydrolysis rate of starch to sugars; (2) retard the hemicellulose hydrolysis rate; (3) reduce tannin oxidation in the skin to avoid chill colours; (4) increase tannin precipitation in the pulp to reduce astringency; (5) decrease the rate of water loss from the skin. To achieve (1) low temperature alone is clearly adequate for most purposes, since it requires approximately 60 days at 53° F. for the starch and sugar levels to attain those of 'eating-ripe' fruit. For (2), reduction to a temperature of 53° F. is inadequate alone; some additional means of decreasing the hemicellulose hydrolysis rate are required. It is possible that such an effect may result either directly or indirectly (as by changing the pH of the tissues) from lowering the external partial pressure of oxygen or from increasing that of carbon dioxide or from both. (3) Oxidation of tannins in the skin may be reduced by lowering the external partial pressure of oxygen (Wardlaw, 1940). It is possible that the effect required in (4) may be achieved by varying the oxygen and carbon dioxide concentrations of the external atmosphere in such a manner as to induce a larger measure of anaerobic respiration within the pulp tissue, aldehydes formed in the intermediary stages of this process may conceivably combine with the tannins. (5) The excessive water loss from the skin, the effect of which is so apparent on the pulp/skin weight-ratio, may be reduced by increasing the humidity or decreasing the air circulation in rooms in which bananas are stored and ripened, or by some waxing treatment. It should be noted that the storage room used for the present work had a lower average relative humidity than that normally attained in commercial practice. The effects of varying the relative humidity require investigation in relation to the other factors of storage and ripening.

Any differences in ripening behaviour between fingers attached to the main stem and detached as clusters also deserve investigation in view of the trade in such 'specials'.

XII. Summary

1. The changes in the amounts of dry matter, starch, sucrose, glucose, fructose, glycosidic-glucose, and titratable acidity have been followed in the pulp and skin of detached bunches of Gros Michel bananas of commercial grade during storage at 53° F.

2. The fresh weight of whole fingers fell fairly steadily throughout the entire period of 99 days. The fresh weight of the pulp fell slightly at first;

after 20 days it rose, reaching a peak value at approximately 60 days; it then fell during the remainder of the period. The fresh weight of skin fell continuously but most rapidly between 20 and 40 days.

3. The pulp/skin weight-ratio was constant for the first 20 days, then rose to a peak value on day 75, falling values then being recorded. The value of 2·0 for this ratio, associated in the literature and previous papers in this series with the 'eating-ripe' stage of the banana, was attained earlier than the stage indicated by the carbohydrate composition as 'eating-ripe'.

4. The percentage amount of dry matter in the pulp changed little during the first 20 days and then fell. After 40 days the rate of fall was slow and steady. The starch percentage changed little during the first 20 days, after which the decrease characteristic of the pulp of ripening bananas occurred, requiring, however, in this instance approximately 40 days for completion instead of 7 as at tropical temperatures. Total sugars rose in percentage amount concurrently with the starch percentage fall. The drifts of the percentage amounts of sucrose, glucose, fructose, and titratable acid are on an extended time basis similar to those in bananas at higher temperatures. The high glycosidic-glucose concentrations previously observed can, apparently, only be developed during ripening at a higher temperature which follows storage at 53° F. sufficiently long to produce chilling.

5. In the skin the percentage of dry matter also changed little during the first 20 days and then, due to water loss, rose consistently. The starch percentage fell until the 44th day and then remained almost constant. Total sugars increased in percentage amount steadily over the entire period. Other analytical data, as for the pulp, are given and discussed.

6. The data are also expressed as absolute amounts in the pulp and in the skin of a single finger, and the differences resulting from the two methods of expression are discussed.

7. After the 37th day the absolute amount of dry matter in the skin increased at the expense of the pulp. The added dry matter was apparently in the form of sugars and hemicellulose.

8. From the drifts of the total amounts of dry matter in pulp and skin the weekly rates of loss of dry matter by the whole finger were calculated. Assuming that all dry matter lost was utilized in respiration, the respiration rate drift may be followed for the whole finger. The general form of the drift agreed with that obtained in studies of carbon dioxide production. The considerable discrepancy between deduced and observed values is discussed.

9. The differential effects of temperature on the metabolic processes of the banana are considered, and suggestions, based on present knowledge and hypotheses, are made for future investigations on storage and ripening.

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Contributions to the Cytology of Spermothamnion Turneri (Mert.) Aresch.

II. The Haploid and Triploid Generations

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With Plate I, and one Figure in the Text

I. INTRODUCTION

PART I of this paper (Drew, 1934) was in the main a description of diploid plants found at Woods Hole, Mass., U.S.A., and the reproductive organs that develop on them. No haploid plants were found in the material then examined, although there was some indirect evidence of their existence. The diploid plants were found to give rise to normal haploid tetraspores (capable of germinating) and in addition to sexual organs. Cystocarps matured as a result of the fertilization of the diploid carpogonia, and these when examined cytologically were found to be tetraploid or very rarely triploid.

Since the publication of Part I of this paper further American plants have been examined and, as it seemed desirable to investigate the behaviour of the species in a completely different locality, some from the British coast[1] as well.

Whilst the investigation was not carried as far as was originally hoped, the facts ascertained are of sufficient importance and interest to justify publication. Chief among these is the establishment of the existence of triploid plants on both sides of the Atlantic Ocean and of haploid plants on the British coast.

The account of these observations falls into two sections, the first dealing

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[1] In using the name *Spermothamnion Turneri* (Mert.) Aresch. for the American material, opinion was reserved as to its identity with the European material for which the same name was used. As a result of this further work, the writer now considers the material on the two sides of the Atlantic to belong to the same species. Plants collected at different times in the growth season from the same locality have very different appearances. It seems highly probable that *Spermothamnion Turneri* (Mert.) Aresch. and *Spermothamnion roseolum* (Ag.) Pringsheim are not separate species.

with the further investigation of the American material and the second relating
to the British material.

The same technique as that used previously was employed for this work.

II. Spermothamnion Turneri at Woods Hole, Mass.

Two questions of considerable importance were left unanswered at the time
of the publication of the first part of this paper, namely the fate of the tetra-
ploid and triploid carpospores and the possible existence of a haploid genera-
tion. After examination of considerable amounts of new material very little
fresh evidence in answer to these questions can be given except in the case
of the triploid carpospores from which, it is legitimate to presume, the newly
discovered triploid plants develop.

Filaments, the nuclei of which show the triploid number of chromosomes,
occur fairly commonly in material collected at the end of April 1935. They
differ from diploid filaments present at the same time by the slightly larger size
of both the filaments and the nuclei in them. The nucleolus is often lobed as is
shown in Pl. I, Fig. 1, which is a drawing of one of these triploid nuclei.

With the exception of a single filament, on which had developed a procarpic
branch since degenerated, these plants are sterile, as are other plants at this
time of year. The triploid may possibly reproduce vegetatively by fragmenta-
tion of the thallus and there is some evidence that tetrasporangia develop, as
filaments have been found with nuclei having between 45 and 50 chromo-
somes, that is, approximately half the triploid number. (Plate I, Fig. 2.) These,
like the triploid plants, occur in April.

It seems doubtful whether there is a geographical segregation of the genera-
tions similar to that found by Papenfuss (1935) in the case of *Ectocarpus sili-
culosus* in this neighbourhood, as a collection from a more outlying region was
uniformly diploid. It was thought that the fact that Woods Hole is at the
northern end of the range of this species might explain the absence of the
haploid and so an attempt to collect material from a lower latitude was made
in 1937. This was unfortunately unsuccessful.

With the possibility of a seasonal distribution of the generations in mind,
collections have been made over as many months as possible. These include
material fixed in April, May, June, July, August, and September, covering the
greater part of if not the entire growth season. Diploid plants are to be found in
all of these collections and triploid plants in April. Haploid plants have never
yet been found.

It should be emphasized that although there is still no direct proof of the
presence of haploid individuals at Woods Hole there is a certain amount of
evidence that they occur and function. The presence of a haploid nucleus in a
trichogyne and a triploid gonimoblast were put forward in favour of this view
in the earlier paper, and this is supported now not only by the occurrence of
triploid plants but by another example of a haploid nucleus in a trichogyne. It
is significant these records refer to material collected at well-separated inter-
vals, namely 1927, 1935, and 1936.

In Part I of this paper spermatangia were recorded on the American coast for the first time. This extended examination has confirmed the suspected scarcity of spermatangial plants in this neighbourhood, but it has shown that they occur over a considerable part of the growth season, namely, May, June, and August. In one case the plant bearing the male reproductive organs was known to be diploid, but the chromosome complement of the other plants bearing spermatangia is unknown.

III. Spermothamnion Turneri in Britain

Most of the British collections have come from localities in Anglesey but others are from Devon, Dorset, and Pembroke. Together they cover the complete season of active growth, that is from March to September, and were made between the years 1932 and 1938. The height of the reproductive period, both sexual and asexual, is in June.

Cytological investigations have shown that haploid, diploid, and triploid individuals occur but, as at Woods Hole, the diploid plants are the most common. Diploid plants bear tetrasporangia and there is a reduction division prior to the formation of the spores. They bear healthy procarps in addition, but these are produced neither so constantly nor so numerously as on the American diploid plants. Although procarps occur, neither cystocarps nor spermatangial branches have been seen on individuals known to be diploid. However, a spermatangial branch has been seen on a filament bearing maturing tetrasporangia, this being therefore in all probability a diploid plant. Pl. I, Fig. 3 shows a diploid nucleus of British material.

Haploid plants are never very common and appear most frequently in the collections made in the early part of the growth season, March, April, and June. Pl. I, Fig. 4 shows a haploid vegetative nucleus. Sexual reproductive organs occur regularly on these haploid plants and begin to be formed when the plants are quite young. Cystocarps result from the fertilization of the carpogonium, and Pl. I, Fig. 5 shows a haploid nucleus of a cell of an enveloping filament of a cystocarp.

There is nothing to add to the description of the procarpic branches and the development of the cystocarp given in the earlier paper, but it is now possible to state that the spermatangial branches are similar to those of *Spermothamnion Snyderae* (Drew, 1937).

All nuclei seen in division in the cells of the spermatangial branches have the haploid number of chromosomes, and one of these is figured (Pl. I, Fig. 6). Little has been ascertained about the chromosome content of the nuclei of the developing procarps and gonimoblasts. The carpospores are usually uninucleate but occasionally they divide. In the prophase of one such division the triploid number of chromosomes was counted (Pl. I, Fig. 9), thus confirming the supposition that the formation of triploid plants is a fairly common occurrence. Sporangium-mother-cells have occasionally been seen on plants known to be haploid. Some of these were healthy but others degenerate, none having undergone division.

Triploid plants have been found in England in a collection made in Dorset in April 1938; this is the same time of year when the triploids appear at Woods Hole. Like the American triploids they are sterile and also show the increased size of both cell and nucleus. The nucleus is more nearly spherical, however (Pl. I, Fig. 7).

As in the case of the American material plants appear with a chromosome complement varying in number from 45 to 50. It seems likely that, although all the triploids seen have been sterile, they do occasionally form tetra-spores, an irregular division preceding their formation. In this connexion the occasional occurrence of tetrasporangia on the triploid of *Plumaria elegans* (Drew, 1939) should be recalled. Unfortunately, most of these nuclei with 45 to 50 chromosomes were seen on poor preparations, but it has been possible to figure the clearest (Pl. I, Fig. 8).

An interesting abnormality, similar to some of those reported for *Spermothamnion Snyderae*, has appeared. A multinucleate cell with the nuclei in prophase has one with the diploid number (Pl. I, Fig. 10) of chromosomes and the rest with the haploid number (Pl. I, Fig. 11).

Binucleate sporangium initials occur and these give rise to the polysporangia previously reported.

IV. Discussion

After the discovery of reduction division in *Osmunda regalis* and other cryptogams, the nuclear cycle in the remaining plant groups became the subject of investigation, as was only to be expected. The first of the Florideae to be examined were species with tetrasporic as well as sexual individuals, and the investigations showed that the tetrasporic plants were all diploid, that reduction division took place prior to the formation of the tetraspores which were consequently haploid, and that these gave rise to haploid plants bearing sexual organs. Considerably later a species with no tetraspore-bearing plants was investigated by Svedelius (1915), who found that reduction division took place immediately after fertilization. The sexual individuals were therefore haploid and the diploid phase extremely short. As these life cycles were some-what different from ones already known, Svedelius suggested a new terminology and proposed that the former type of life-cycle should be called diplobiontic and the latter haplobiontic. Looked at from the older and strictly morpho-logical point of view there are three generations or phases in the diplobiontic types and two in the haplobiontic. In the former case these are represented by:—(1) the sexual plant or plants, (2) the cystocarp, and (3) the tetraspore-bearing plant. Kniep (1928) refers to these as the gametophyte, the carpo-sporophyte, and the tetrasporophyte respectively. In the haplobiontic types only the first two generations or phases appear. In both types there is an alternation of haploid and diploid nuclear phases, but whereas in the haplo-bionts the diploid phase is reduced to the minimum, in the diplobionts the two nuclear phases are of more equal duration, the diploid consisting of two spore-bearing generations. One other point to notice is that while morphologically

the cystocarp is homologous in both the haplo- and diplobionts, in the first group it is haploid and in the other diploid. It would seem, therefore, that the cystocarp has arisen by a development of the fertilized egg cell and has been interpolated, but the tetrasporophyte has originated by a postponement of reduction division.

From certain morphological evidence it was obvious that there must be exceptions to these two generally accepted types, and of the species bearing sexual organs and tetrasporangia on the same filaments, *Spermothamnion Turneri* was perhaps the best known. The present investigation was undertaken in the hope of elucidating this deviation from the 'normal'. It has shown that it is due to the occurrence of sexual organs on tetrasporangium-bearing diploid plants in addition to the usual haploid plants. While no evidence has accrued suggesting that asexual spores mature on the haploid as occasionally happens in *Nitophyllum punctatum* (Svedelius, 1914), for example, healthy as well as degenerate sporangium-mother-cells have been seen. The sexual organs on diploid plants are diploid, and as a direct consequence of the occurrence of both haploid and diploid sexual cells triploid and tetraploid carpospores are produced in addition to the usual ones which are diploid. The triploid as well as the diploid carpospores appear to be viable, triploid plants having been found on both sides of the Atlantic Ocean. There is no evidence yet of tetraploid plants, which if produced, might well reproduce the diploid and thus account for its preponderance numerically.

As was pointed out in Part I of this paper (1934), the morphological structures produced do not depend on the cytological state of the individual as indeed might be expected from the existence in the Florideae of cystocarps morphologically homologous but sometimes haploid and sometimes diploid. It seems to be the diploid which shows the greatest latitude, and certain facts suggest that this may depend somewhat on environmental conditions.

The present state of our knowledge regarding *Spermothamnion Turneri* can perhaps be summarized best by the following diagrams (Text-fig. 1). Where only indirect evidence is available, this is indicated either by a dotted line or italics. The author wishes to emphasize that this diagram is not in any way a life-cycle diagram. This is particularly true in the case of the American material where there is no evidence as to how the diploid condition is maintained, whether by vegetative means or by sexual fusion of haploid cells or by reduction division from a tetraploid. Further investigations on the American coast are very necessary. One fact not brought out by these diagrams is the great rarity of spermatangia on American plants and that when produced these male branches are very poorly developed.

The only Floridean alga for which triploid individuals have been reported previously is *Plumaria elegans* (Drew, 1939). Whereas their method of origin remained undiscovered in the case of *Plumaria elegans*, this has been established in the case of *S. Turneri*. The triploid of *P. elegans* has become independent by the development of a particular kind of spore, the paraspore, which has enabled it to spread both rapidly and extensively. The triploid of

AMERICAN MATERIAL

Diploid plants (60).

Procarps (60).

Carpogonia (60).

→ Fusion nucleus.

Gonimoblasts (90).

Carpospores.

Triploid plants (90).

Sporangia with R.D.

Plants with 45–50 chromosomes.

Spermatangial branchlets.

Spermatangia.

Fusion nucleus.

Gonimoblasts (120).

Carpospores.

Tetraspore-mother-cells.

Tetraspores (30).

Germlings (30).

Haploid plants.

Haploid spermatangia.

Procarps (degenerate).

BRITISH MATERIAL

Haploid plants (30).

Procarps.

Carpogonia.

Spermatangial branches (30).

Spermatangia (30).

→ Fusion nucleus.

Gonimoblasts (60).

Carpospores.

Diploid Plants (60).

Procarps.

Carpogonia.

Spermatangial branches.

Spermatangia.

Fusion nucleus.

Gonimoblasts.

Carpospores (90).

Triploid plants (90).

Sporangia with R.D.

Plants with 45–50 chromosomes.

Tetraspore-mother-cells.

Tetraspores.

TEXT-FIGURE. Illustrating in diagrammatic form the present state of our knowledge regarding *Spermothamnion Turneri*. The figures in brackets refer to chromosome number.

S. Turneri, however, does not appear to fit into the life-cycle nor to reproduce itself; but while direct evidence is lacking there is reason for supposing that tetraspores are formed, for plants occur the nuclei of which have approximately half the triploid number. Further investigations regarding polyploidy in other Florideae are highly desirable.

The question whether *S. Turneri* is to be considered as a diplobiont arises. Svedelius (1915) originally defined haplobiontic as meaning the occurrence of only one kind of individual or 'biont' in the life-cycle, and diplobiontic as the occurrence of two individuals. In an amplification of this terminology in 1931 he classes as diplobiontic plants in which the 'two kinds of individual' or generations are either alike or dissimilar morphologically, and stated moreover that 'the two kinds of bionts or individuals need not necessarily coincide with the two cytological generations', as for example in the Florideae. From this it appears that for Svedelius the unit or biont is the physiological unit irrespective of its morphology or cytology; e.g. the Bryophyta are haplobionts. From this the reader is also led to conclude that Svedelius does not regard the cystocarp as a morphological unit. The definition was made when cytological variations such as those of *P. elegans* and *S. Turneri* were unknown, so an emended definition now seems desirable. The present writer would prefer that the terms were kept for the Florideae and based on cytological criteria by which *P. elegans* would be a triplobiont and *S. Turneri* a polybiont.

In comparing the available facts about the behaviour of *S. Turneri* at Woods Hole and in Britain the most obvious difference is in regard to the haploid generation. In Britain it occurs but it is less frequent than the diploid. Up to the present there is no direct evidence of its occurrence at Woods Hole. There also seems to be a stricter segregation of sexual and asexual reproductive organs on the British plants. In spite of these differences the important point of similarity in need of emphasizing, and the justification of publication at this juncture, is that this plant shows a greater variety of lines of development than has been previously found in the Florideae.

V. Summary

1. Plants of *Spermothamnion Turneri* from British shores as well as further collections from America have been examined.

2. Triploid plants occur in April on both the British and American coasts. The cells and the nuclei of these triploid plants are larger than those of the diploid. Although the triploids seen have been sterile, plants with approximately half the triploid number also occur in April and it is suggested that these originate from spores developed on the triploid after an irregular reduction division.

3. Haploid individuals have been found on the British coast although not as frequently as the diploid. They bear sexual organs and occasionally healthy or degenerate undivided sporangia.

of the Marine Laboratory at Woods Hole who helped in the collection of some of the material. The earlier American material was collected while the investigator held a Commonwealth Fund Fellowship.

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EXPLANATION OF PLATE I

Illustrating Kathleen M. Drew's paper on 'Contributions to the Cytology of *Spermothamnion Turneri* (Mert.) Aresch. II. The Haploid and Triploid Generations'.

The nuclei figured are all in late prophase. The drawings were made by superimposing camera-lucida tracings of the chromosomes of successive focal planes. In order to suggest their position in a sphere the chromosomes are variously shaded, those nearest the observer being black and those farthest away white. Where chromosomes are completely behind the nucleolus or other chromosomes no attempt is made to show them. ($\times 3,300$.)

A. American material.

Fig. 1. Triploid nucleus from apical cell of sterile filament. Total number of chromosomes seen 91, 5 of which are behind others or the nucleolus and so not reproduced in the figure.

Fig. 2. Nucleus with 49 chromosomes and one other body (*a*)—which may or may not be a chromosome. Some of these chromosomes are partly behind others.

B. British material.

Fig. 3. Diploid somatic nucleus. Sixty chromosomes present; 6 of these are not shown, being behind others or the nucleolus.

Fig. 4. Haploid somatic nucleus. The structure marked (*a*) may represent one chromosome or else two very close together, bringing the total to 30 or 31. Three of the chromosomes are completely behind the nucleolus.

Fig. 5. Nucleus from cell of enveloping filament of cystocarp. Chromosomes number 29, all visible in the figure.

Fig. 6. Nucleus in cell of developing spermatangial branch. Thirty chromosomes present, one of which is behind the nucleolus.

Fig. 7. Triploid somatic nucleus from apical cell of sterile filament. Of the 91 chromosomes distinguishable, 2 are completely hidden behind others.

Fig. 8. Nucleus with 48 chromosomes, one of which is behind the nucleolus. Filament sterile.

Fig. 9. Nucleus of carpospore in prophase. The chromosomes distinguishable total 86 or 87, depending on whether (*a*) is a single chromosome or two close together. Of the remainder 11 are hidden behind other chromosomes or the nucleolus.

Fig. 10. Nucleus with 54 chromosomes in the apical cell, the remaining nuclei of which are haploid. Four of the chromosomes do not appear in the figure as they are behind the nucleolus.

Fig. 11. Haploid nucleus from same cell as that of Fig. 10. Twenty-eight chromosomes distinguishable, one of which is hidden behind another chromosome and two others are partly covered by the nucleolus.
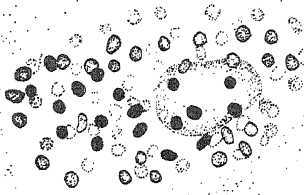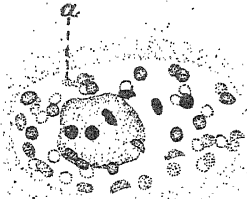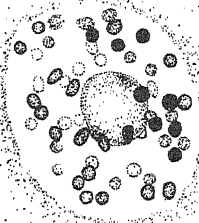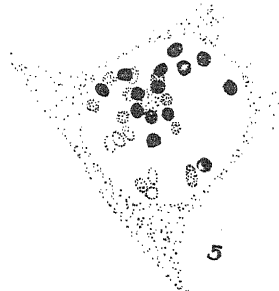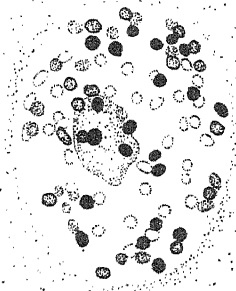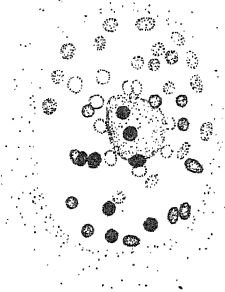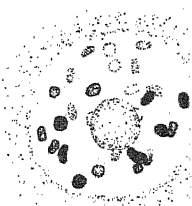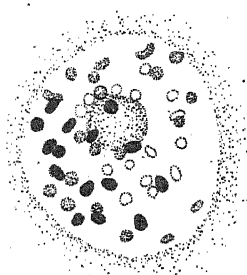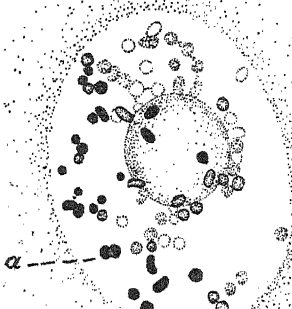
Wilt of Cacao Fruits (Theobroma Cacao)

I. An Investigation into the Causes

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With five Figures in the Text

I. INTRODUCTION

IN many fruit-trees a certain proportion of the crop fails to mature. Some of the fruits are lost through disease, and the development of others is suspended for no clear reason. This cessation of growth or wilting in many cases is accompanied by chemical and anatomical change followed by shedding, whereas, in other cases, cacao being an example, the young fruits wilt and dry up without being shed. The causes of wilting and shedding have been the subject of many investigations on various kinds of fruit-trees with the object of reducing their effects and so increasing the size of the crop. There can be little doubt that there are many different processes involved in the premature shedding, but in general shedding is believed to arise from the operation of adverse physiological factors, internal or external, in particular, water supply and nutrition. The literature of the subject is very extensive, but no attempt will be made here to review it.

In cacao plantations in Trinidad it is common to see young withered fruits of various sizes still attached to the trees, and their failure to reach maturity may be presumed to constitute an important factor determining the magnitude of the crop. Pyke (1932) found that cacao wilt in Trinidad may account for losses of 19 to 92·5 per cent. of the number of fruits set by different trees, while Hewison and Ababio (1929) found losses in the Gold Coast from 22 to 84 per cent.

II. Methods and Procedure

The chief experimental difficulty in investigations on cacao is the heterogeneous genetical constitution of the trees of the average plantation. In the present problem it was desirable to eliminate this factor as far as possible and recourse was made to the nearest approach to mature clonal material available in Trinidad. This consisted of budded and grafted material in the 'colour experiment' at River Estate (subsequently referred to as estate A). The parent material was budded or grafted in 1914 on to stocks whose origin appears not to have been recorded. This material thus provided mature trees growing under typical plantation conditions. In the second year of the experiment modified observations were made on a number of ordinary cacao trees growing in a different part of the island, namely, at La Reconnaissance Estate, Lopinot (estate B), having a somewhat similar sandy-loam soil. In the third and final year of the experiment, true clonal material became available for investigation. The trees of these clones constitute some of the Imperial College Selections (I.C.S.) made by Cheesman and Pound (1933) and Pound (1933), and were grown from rooted cuttings. They were situated at San Juan Estate (estate C) in the Montserrat district of Trinidad, and were 6 years old at the time of the experiment and in their third year of bearing. The soil of this estate is a rich, friable clay-loam, in marked contrast to that in which the other experimental trees were growing. Trees of the same clones situated at estate A were also kept under observation. These were 5 years old at the time of the experiment. The results obtained from all these groups of trees will be discussed in this communication.

The first observations were begun on estate A in March 1939 (during the dry season in Trinidad), when few fruits were setting. The trees were visited weekly and each ovary that had begun to swell, presumably as a result of fertilization, was marked by means of an aluminium label bearing a number. The length and diameter of each fruit were measured, as well as its distance from the base of the main trunk along the branch and the diameter of the branch at the point of attachment of the fruit. In successive weeks besides marking new fruits, measurements of those surviving were also made and their condition noted. In this way it was possible to keep a complete record of the history of each fruit produced throughout a complete growing season. Weekly rainfall data and soil moisture contents at depths of 0–6 in. and 6–12 in. were recorded. Later a Stevenson screen was established in the plot and continuous records of temperature and humidity were thus obtained. Similar observations were made on the other groups of trees already mentioned, but in the case of the ordinary estate cacao trees no measurements of distance of fruits from the ground or of branch diameter were recorded.

From all these observations data were available which could be analysed to determine (a) whether there is a critical stage in the life history of the cacao fruit when it is susceptible to wilt, (b) whether wilting is a function of position on the tree, and (c) whether wilting is related to external conditions.

III. Growth of the Fruit

The growth data are too extensive to be fully presented here, but it was found that when the increases in length of each fruit were plotted against time it was evident that most of the fruits which set in June and July (the beginning of the rainy season in Trinidad) reached maturity or became diseased at a relatively late stage, whereas fruits which set subsequently (i.e. August onwards) wilted before they had attained a length of 10 cm. At the time that the crop which set in June and July was ripening (i.e. about $5\frac{1}{2}$ months later) many of the fruits then setting grew normally and subsequently became ripe. This is illustrated in the case of one tree (estate A: No. 35) for the 1939–40 season in the first column of Table I, where the growth data have been condensed to show the average size attained by fruits setting on any particular date. These include all ripe, diseased, and wilted fruits, and accordingly the average provides only an approximate representation of the true condition. It is clearly shown, however, that fruits which set in June and July attained a large maximum size, whereas those set in August, September, and the beginning of October were still small in size when their growth ceased. At the end of October and during November and a part of December the majority of the fruits became mature and subsequently the maximum size attained fell off again. A striking result which emerged from the large number of measurements of fruit length was that, in the case of estate A trees where the growth data is most extensive, if a fruit reached a length of between 10 and 11 cm. (about 70 days old), it either attained maturity or became diseased at a relatively late stage. After fruits had attained this stage they were never lost through wilt. There were indications that in the case of the trees of estate C this critical size was somewhat larger, a factor which may be correlated with the much greater soil fertility at this site.

IV. Progressive Change in Size of Wilting Fruits during the Season

(a) Mature Trees

It has already been mentioned that there is a progressive change in the average maximum size attained by the fruits set in successive weeks (Table I). This progressive change is more clearly shown when wilted fruits only are considered. In Table II the data obtained from mature trees during three seasons at two different localities are summarized. Against the date of setting are plotted the average size at which the fruits wilted. The average is weighted, allowance being made for the number of fruits from each tree. In the same table is also shown the percentage of fruits which matured from successive weekly settings. It is evident in all cases that the average size at wilting throughout the season diminished progressively up to a certain point. Fruits set at the beginning of July attained a length of about 50 mm. at wilting, but fruits set in October barely attained a length of 20 mm. at wilting

TABLE I

Average Length of Fruits set in Successive Weeks. Estate A: Tree No. 35

Setting date. (Period ending)	Average length attained by fruits. (mm.)	Setting date. (Period ending)	Average length attained by fruits. (mm.)
June 21, 1939	106	Oct. 3, 1939	42
,, 28 ,,	147	,, 10 ,,	41
July 6 ,,	110	,, 17 ,,	93
,, 12 ,,	162	,, 24 ,,	85
,, 22 ,,	159	,, 31 ,,	(25)
,, 31 ,,	98	Nov. 6 ,,	88
Aug. 9 ,,	103	,, 15 ,,	167
,, 15 ,,	82	,, 21 ,,	(18)
,, 22 ,,	91	,, 27 ,,	160
,, 29 ,,	86	Dec. 13 ,,	98
Sept. 6 ,,	77	,, 20 ,,	79
,, 12 ,,	47	,, 27 ,,	82
,, 20 ,,	49	Jan. 3, 1940	71
,, 25 ,,	40	,, 9 ,,	78
		,, 15 ,,	87
		,, 23 ,,	66

TABLE II

Average Size at Wilting of Fruits set in Successive Weeks, and Percentage of Total Set which became Ripe or Diseased, at Two Estates in Different Seasons

ESTATE A

Week of year in which fruit was set.	Average size at wilting. (mm.)			Percent. of total set which become ripe or diseased.		
	Season 1939–40. (Average 13 trees.)	Season 1940–1. (Average 4 trees.)	Season 1941–2. (Average 4 trees.)	Season 1939–40.	Season 1940–1.	Season 1941–2.
July 26	52	—	43	32	—	46
27	51	—	56	30	—	35
28	47	69	52	23	35	17
29	47	56	57	28	42	16
30	51	46	52	32	2	3
August 31	45	40	50	23	1	2
32	43	37	40	14	2	2
33	41	32	35	6	0	0
34	39	29	32	8	0	2
September 35	33	31	25	5	0	2
36	33	29	26	2	0	2
37	27	26	22	2	0	3
38	25	22	29	2	0	2
39	22	21	27	4	0	0
October 40	19	22	17	5	3	6
41	19	19	(12)	13	0	0
42	21	26	67	24	0	14
43	(24)	26	68	26	27	10
November 44	(10)	25	—	47	9	—
45	27	27	51	31	17	25
46	42	79	49	37	20	33
47	—	51	45	45	0	18
December 48	55	48	45	33	6	21
49	38	58	40	22	8	17
50	—	45	41	—	0	10
51	—	55	40	—	6	9
52	—	40	39	—	0	6

ESTATE B

Week of year in which fruit was set.		Average size at wilting. (mm.)		Percent. of total set which become ripe or diseased.	
		Season 1940–1. (Average 7 trees.)	Season 1941–2. (Average 3 trees.)	Season 1940–1.	Season 1941–2.
July	26	—	—	—	100
	27	—	22	—	63
	28	67	(43)	60	50
	29	50	—	28	75
	30	51	48	17	80
August	31	40	32	12	58
	32	35	48	11	34
	33	42	71	12	38
	34	50	54	12	13
September	35	40	46	4	20
	36	45	49	4	10
	37	45	42	6	10
	38	38	31	6	10
	39	37	32	6	5
October	40	40	31	6	0
	41	34	(49)	4	33
	42	30	—	5	—
	43	36	—	0	0
November	44	19	—	8	0
	45	64	—	0	—
	46	(42)	76	33	25
	47	20	—	0	—
December	48	(53)	59	0	14
	49	63	59	0	55
	50	78	53	25	0
	51	41	42	22	23
	52	56	43	13	33

in the case of the estate A trees. The values for the estate B trees were somewhat larger. In the November and December settings the average size at which wilting took place again increased, a time when a larger proportion of fruit then set became ripe. These observations suggest very strongly that fruits which began development early in the season were able to obtain an adequate supply of water and nutrients, whereas the later fruits had only restricted supplies to draw upon, so that the trees were unable to absorb these at a sufficiently rapid rate to satisfy their needs, consequently wilting occurred.

(b) Young Trees

The sequence of events in the case of the young clonal I.C.S. (1) trees at estate A and estate C will be considered separately, because their behaviour is somewhat different from that of mature trees. In Fig. 1 have been plotted data showing the average size at wilting and percentage of fruits becoming ripe of those set in successive weeks for the two sets of I.C.S. (1) trees situated at the two different estates. In both cases there was a rapid diminution in size at wilting after a slight initial rise, reaching a very low value at

the end of September. This was a time when practically all the fruits remaining on the trees wilted, and on both estates at this time the trees 'flushed' heavily, the downward-pointing arrows (Fig. 1) indicating the date
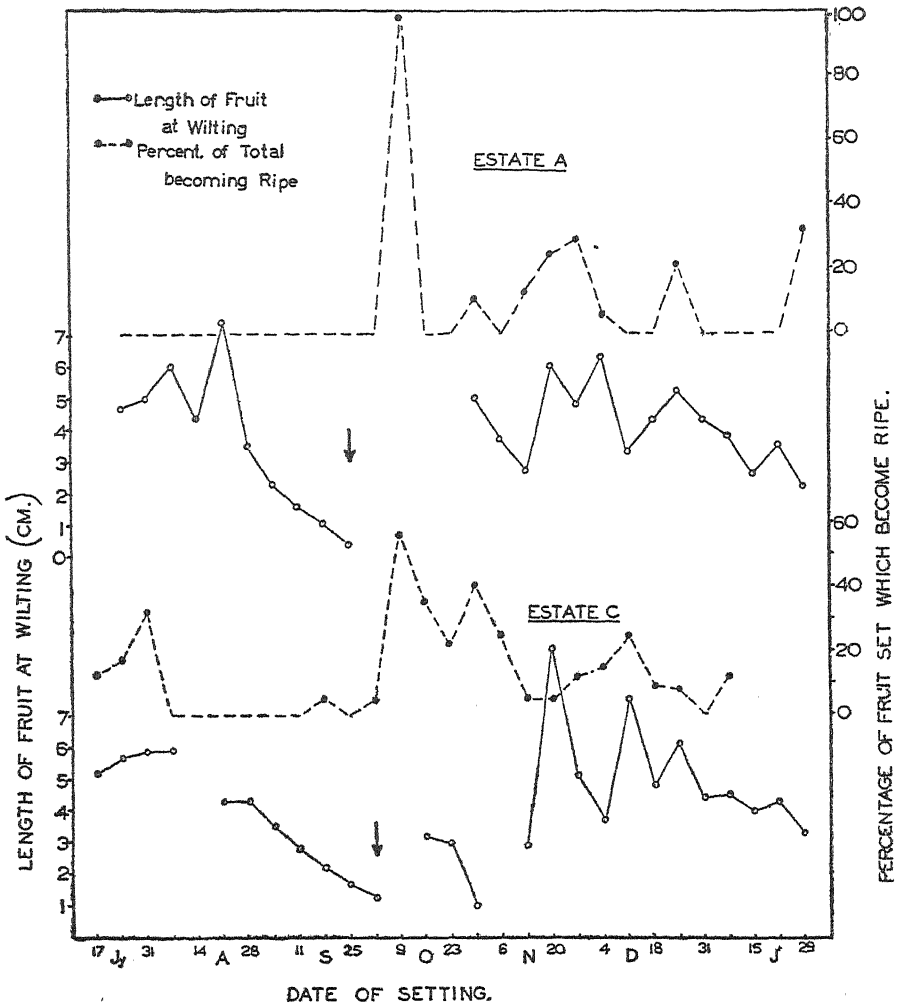
Fig. 1. The average size at wilting of young fruits set in successive weeks (continuous line) and the percentage of the total set which become ripe (dotted line) at estate A and estate C. The data refer to trees of Imperial College Selection No. 1. The downward pointing arrows indicate the time when the flushes of young leaves on the trees were at their maximum.

when the flush of leaves was at its height. There can be no doubt that the heavy change of leaf caused considerable loss of young fruits. None of the fruits set on the estate A trees in this initial period survived, and only a few matured of those set at the beginning of this period on the estate C trees. Of the fruit set immediately after the flush, however, some became ripe.

This was especially marked in the case of the estate C trees. The size at wilting also again increased and then diminished as the crop on the tree matured. There were marked fluctuations in the size of wilting in this second period, in contrast to the rather regular values for mature trees. This, together with the fact that these young trees were so markedly affected by a leaf-flush, illustrates clearly that they are much more affected by sudden changes in distribution of nutrient materials, probably because they have much smaller reserves to draw upon than mature trees. Consequently, they are also much more susceptible to adverse changes in environment than old established trees. The fact that a large proportion of the fruits set immediately after the leaf-flush reach maturity also suggests that the general nutritional status of the tree is enhanced by the more efficient carbon assimilation of the large number of young leaves then present.

V. EFFECT OF POSITION OF THE FRUIT

It has been tentatively suggested that the ability of a cacao fruit to survive is a question of its obtaining a continuous adequate supply of nutrient materials. It is therefore likely that the position of the fruit on the tree will be important. Since records of distances of the fruits from the ground measured along the trunk and branches and of the diameters of the branch at the point where the fruits are borne were obtained in the case of every fruit set on the experimental trees at estate A, during the first year of the investigation, it is possible to analyse the data from that point of view. The data are summarized in Table III. Only fruits set during the 8 weeks' period

TABLE III

Comparison of the Position on the Tree of Ripe and Wilted Fruits set during the Period June 25 to August 19, 1939

ESTATE A

Number of tree.	Average distance from ground of ripe and diseased fruits. (cm.)	Average distance from ground of wilted fruits. (cm.)	Level of significance of difference between distances. (P)	Average diameter of branch at point of attachment of ripe and diseased fruits. (cm.)	Average diameter of branch at point of attachment of wilted fruits. (cm.)	Level of significance of difference between diameters (P)
31	247	294	< 0·05	4·8	2·5	< 0·01
32	376	423	< 0·05	3·9	1·9	< 0·01
33	197	302	< 0·05	4·8	2·8	< 0·01
34	214	331	< 0·05	5·0	2·8	< 0·01
35	347	379	not sig.	4·4	3·0	< 0·01
319	278	311	not sig.	5·3	3·6	< 0·01
320	339	398	< 0·05	5·4	3·6	< 0·01
321	257	276	not sig.	4·8	4·1	not sig.
322	165	272	< 0·01	5·8	4·1	< 0·05
893	298	361	< 0·01	4·9	2·5	< 0·01
895	208	359	< 0·01	7·0	3·1	< 0·01
896	281	420	< 0·05	6·8	3·0	< 0·01

(June 25 to August 19) are included in this analysis, since during that period only were there enough data to warrant statistical examination. In the case of every tree the average distance from the ground of ripe and diseased fruits was less than that of wilted fruits. Similarly, the average diameter of the branch at the point at which ripe and diseased fruits were borne was in
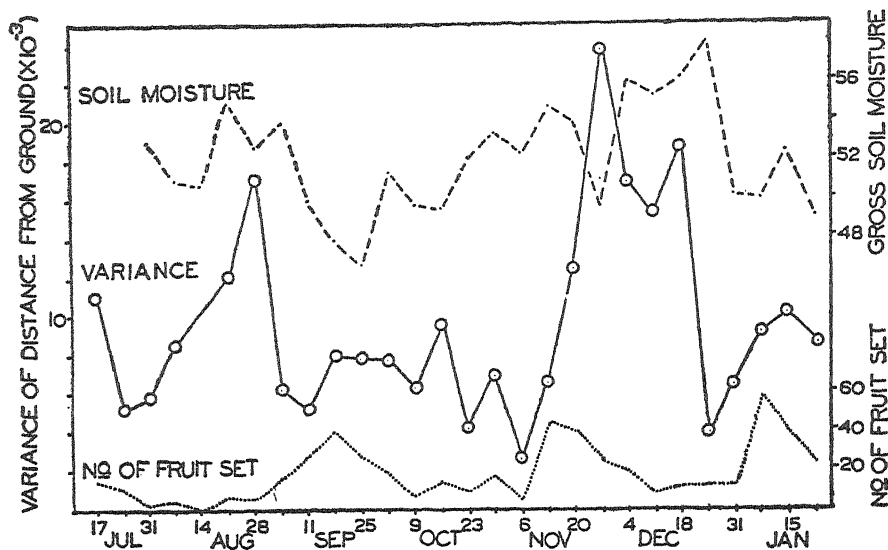
FIG. 2. Successive changes in variance of distance of fruit from the ground, number of fruit set, and gross soil moisture at the experimental plot on estate C (I.C.S.1. trees). Season 1941–2.

all cases greater than that of the branch at the point at which wilted fruits were borne. In all cases (except for trees 35, 319, and 321) the difference between the distances was significant, and only in the case of tree 321 did the difference between the branch diameters fail to be significant. The reason for these exceptions is not immediately apparent, but they might be explained by the particular shape of the trees. It is clear, then, that fruits which set on the thinner branches, and are therefore situated farther from the ground, are more susceptible to wilt than those set on thicker branches nearer the ground. In this connexion it may be noted that Pound (1931) has shown that cacao fruits are smaller the farther they are from the ground, and that Esbjerg (1934) has shown that higher yields are obtained from apple trees having trunks 0·5 m. high than from those having trunks 1·0 m. high growing under similar conditions.

Having established that the position of fruits on the cacao tree is an important factor in their survival, it is of interest to examine in what manner the fruits vary in position on the tree throughout a season, and to investigate the factors that determine their distribution. In the case of three of the groups of trees the variance (i.e. the square of the standard deviation) of distance from the ground of weekly sets has been calculated. These data are plotted in Figs. 2, 3, and 4. In all cases there is a clear trend in the variance

from week to week. The value is low at the beginning of the wet season but rises in the next few weeks to a maximum value and then falls again. In the case of the estate A trees (Figs. 3 and 4) the variance values subsequently rise to two secondary maxima in August–September and October, while in the case of the estate C trees (Fig. 2) a second maximum occurs in November–
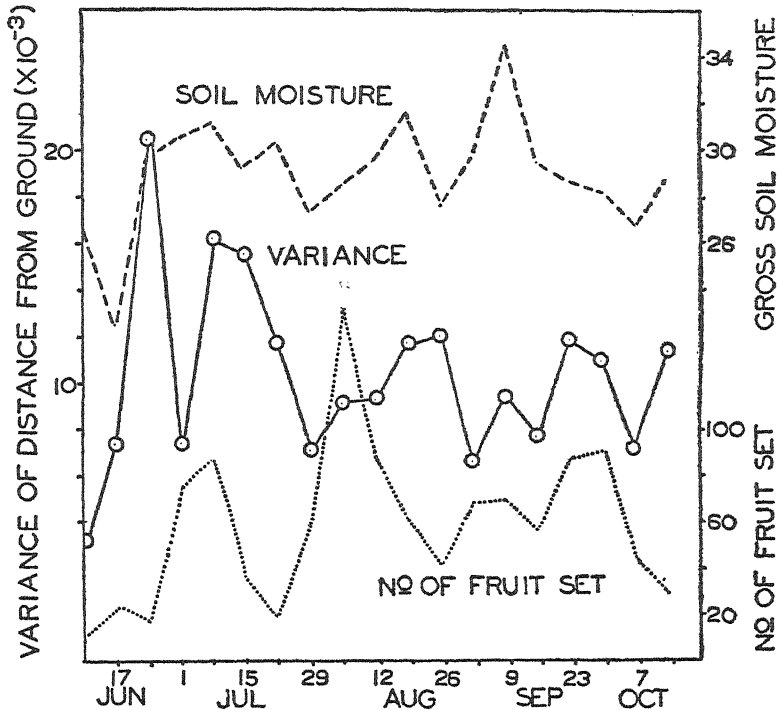
FIG. 3. Successive changes in variance of distance of fruit from the ground, number of fruit set, and gross soil moisture at estate A: trees 31–5. Season 1939.

December, the values being even higher than in the first maximum. That the value for variance is independent of the number of fruits set in particular weeks is also shown in the diagrams (Figs. 2, 3, and 4) where the numbers of fruits set in successive weeks are plotted, so that it is assumed that the calculation of variance gives a true measure of the scatter of new fruits on the tree for successive weeks. It seems probable that one of the chief factors influencing the position of the fruit is the moisture conditions in the air or in the soil or both, a probability which is borne out in Figs. 2, 3, and 4, where the gross soil-moisture values at a depth of 0–12 in. have also been plotted. This depth was selected since it is believed that the chief absorbing roots of the cacao tree are situated at this level. The soil-moisture curve shows a somewhat similar trend to the variance curve. During or following a period of high soil moisture the variance is usually high, whereas a drying-out of the soil appears to cause a decrease in the variance, or, in other words,

the fruits are restricted in position on the tree. The data plotted in Figs. 2, 3, and 4 refer to two different seasons and to two different soils, so that the relationship between variance and moisture conditions appears to be fairly close, although it is somewhat unfortunate that the two seasons are rather similar in that there was a comparatively dry spell in the middle of each
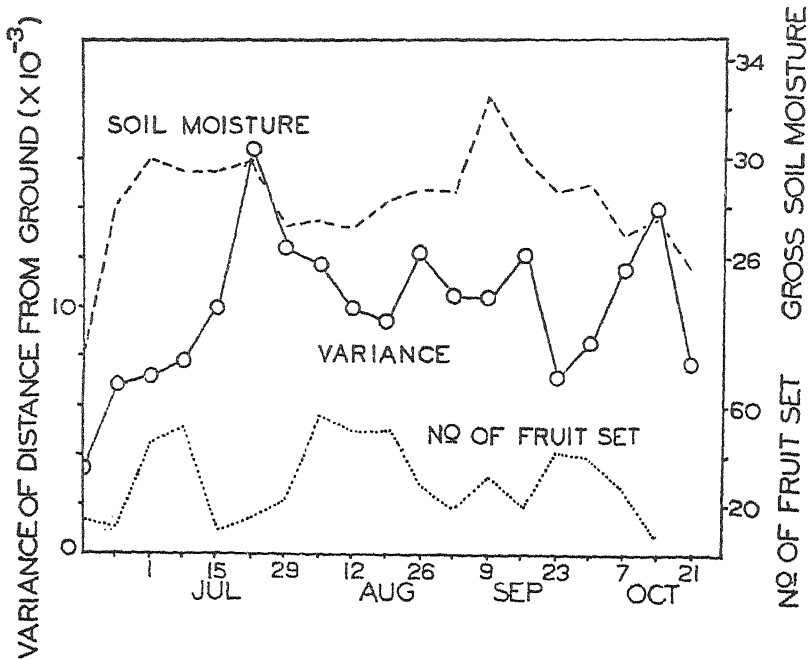
FIG. 4. Successive changes in variance of distance of fruit from the ground, number of fruit set, and gross soil moisture at estate A: trees 318–22. Season 1939.

observation period. It would be desirable to discover if this relationship holds over a wider range of environmental circumstances. Under conditions obtaining in Trinidad, however, it is usual for a fairly dry spell to occur in September or October. In the case of the estate C trees (Fig. 2) it is noteworthy that an unusually long dry spell during this period was followed by a rather wet spell, and this was reflected in the variance values which rose and fell again in a regular manner over a period of 7 weeks. The conditions before and during this period were somewhat similar to those occurring at the end of the dry season and the beginning of the wet season in a normal year, and apparently they exerted a similar effect on the distribution of the fruits on the tree. It appears therefore that, during periods of high soil moisture, more fruits are set on the thinner branches. The data presented do not necessarily indicate that the soil moisture alone is the chief factor influencing the distribution of fruits on the tree, since the gross soil-moisture curve may be regarded as a smoothed curve of rainfall conditions over the period, and it is possible that the moisture status of the atmosphere is also

important. In order to test this possibility it would be necessary to vary the humidity of the air and soil independently. It seems likely, however, that potential supply of water and nutrients will be the most important factor influencing position of fruits on the tree.

VI. MINERAL UPTAKE OF DEVELOPING FRUITS DURING A COMPLETE SEASON

The evidence so far adduced is mainly circumstantial, but it suggests that the cause of wilt of young cacao fruits is primarily competition for water and nutrients. More direct evidence, however, has been obtained. The growth-in-length measurements of all experimental trees at estate A (1938–9 season) provide an accurate guide to the size of the crop, and hence of the extent of the demand by the fruit on the trees at any particular time. If the mineral requirements of a fruit at any stage of development are known, it is possible to estimate the mineral uptake at regular intervals. An investigation of the mineral requirements of developing fruits on trees of the same variety and growing on the same soil as those used in the present experiment will be reported in a subsequent paper. Meanwhile some of the analyses have been utilized in conjunction with the growth data to give an estimate of the potassium uptake (expressed as K_2O), at approximately fortnightly intervals, of the crop on five trees (estate A, Nos. 31–5) during a complete season. Potassium was selected as an example, not because it is necessarily more important than other elements but because it is the element most in demand by the fruit. The amounts for other elements run more or less parallel to the potassium figures. The results are presented graphically in Fig. 5. In the same figure has also been plotted the average size at wilting of fruits *set* in successive weeks on these particular trees. At any point the horizontal arrows indicate the average survival time of fruits set in a particular week. It is evident that most of the young fruits tend to wilt during the period when the maximum demand for mineral elements is made on the tree, and that fruits set at this time have the shortest life.

VII. DISCUSSION

As a result of his studies of fruitfulness in cacao Pound (1931a) concluded that weather plays a large part in wilting; he pointed out that when no fine spell occurred in October wilt was most prevalent. The detailed observations extending over a period of three years summarized in the present paper seem to lend no support to this view. Pound also regarded incomplete pollination or incompatibility as possible causes of wilt. The former was eliminated by his observation that there was an insignificant difference between the numbers of ovules in wilted and sound fruits. That incompatibility may be an important factor seemed more probable, since Rounce and Smart (1928) had observed that maximum wilting occurred at the same time after pollination as Cheesman (1927) had indicated to be coincident with the first division of

POTASSIUM   UPTAKE (Grams K_2O.)

Size at wilting. Cm.

FIG. 5. Increments of potassium uptake of the developing fruits (expressed as K_2O), at approximately fortnightly intervals, throughout a year on 5 trees of estate A. The average size at wilting of young fruits set in successive weeks is also shown. The horizontal arrows indicate the average expectation of life of young fruits and the points of the arrows denote the average date at which fruits set in any particular week would wilt.

the zygote. In the present investigation, however, it was found that wilting took place in fruits of all sizes up to about 10 cm. length (approximately 70 days old), and that the size at which wilting occurred varied throughout the year. It will be demonstrated in a later paper, however, that at the time of the division of the zygote important biochemical changes take place. Pyke (1932) found that in general there was an increase of wilting at the time of, or immediately following, each leaf-flush. This was not observed in the present investigation in the case of mature trees, but was especially marked in the young I.C.S. trees of 5 and 6 years old. A heavy flush sometimes caused the loss of all fruits of less than 10 or 11 cm. length. This fact affords additional support to the present hypothesis that wilt is primarily caused by an insufficiency of mineral nutrients, since the simultaneous development of a large number of leaves makes considerable demand on the nutrient reserves of the tree. The nature of this demand for nutrients has been studied by analyses of flushes at weekly intervals from the time that the buds burst (Humphries, 1939), and it is shown that development of a new flush may cause considerable drain on the mineral nutrients from older leaves.

Voelcker (1936) has collected data regarding fruit-shedding in Forastero cacao in Nigeria. He followed the development of fruit from hand pollinations made at intervals during the course of a whole year. He found that most fruits resulted from pollinations made during the early rains (April–June), which agrees with the results of the present investigation. He also noted a second period (December) when a large percentage of the flowers pollinated yielded ripe fruits, an observation which again falls into line with the present results since his figures show that the second crop set approximately 6 months later than the first crop, or at a time when the first crop was mature. Voelcker also found that the age at which shedding took place varied throughout the season, and a re-examination of his figures shows that fruits produced when the main crop was setting had a longer 'expectation of life' than those set when the main crop was maturing. It was found that the maximum age at which shedding took place was about 80 days, which is not very different from the 70 days stressed in the present paper. Voelcker (1937) carried out a similar investigation in Trinidad with comparable results. All Voelcker's results closely support the present hypothesis as to the cause of cacao wilt.

Srivastava (1938) came to a similar conclusion in the case of premature dropping of pear fruits. He showed that fruits that fall early are the smallest in size at the start. Fruits hanging longer and those which reach maturity are larger at the start, and grow much more rapidly every week than those which have a shorter life on the tree, indicating that shedding of fruits arises from competition for nutrients.

VIII. Summary

1. An investigation, extending over a three-year period, has been made into the factors concerned in cacao fruit wilt. Mature budded and grafted

trees, ordinary estate trees, and young clonal material from the Imperial College Selections were used.

2. The following evidence is adduced in support of the theory that cacao wilt is primarily due to nutrient and water deficiency. (*a*) Fruits which set early in the season reached maturity or became diseased at a relatively late stage, but fruits which set later usually wilted. When the crop began to ripen, many of the fruits then setting did not wilt and eventually became ripe. (*b*) As the season progressed, the size at which the fruits wilted became progressively smaller until the crop matured, after which the size at wilting became larger again. (*c*) Fruits on the thinner branches, and therefore farther from the ground, were more susceptible to wilt than those on the thicker branches. (*d*) Most of the fruits tended to wilt during the period when the maximum demand for mineral nutrients is made on the tree by the developing crop. (*e*) Young trees were particularly affected by a heavy flush of new leaves which caused developing fruits to wilt.

3. Moisture relations of the soil and atmosphere appeared to be important in determining the position of fruits on the tree. During and following periods of high soil moisture the variance of distances from the ground of setting fruits was high, i.e. fruits were more scattered over the tree.

4. No evidence was found that weather had any direct effect on the incidence of wilt, although any adverse conditions tending to accentuate the competition for nutrients would be expected to result in increased wilting, particularly in young cacao trees.

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Wilt of Cacao Fruits (Theobroma Cacao)

II. A Preliminary Survey of the Carbohydrate Metabolism with Special Reference to Wilt Susceptibility

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With five Figures in the Text

I. INTRODUCTION

IN the course of a preliminary investigation (Humphries, 1942) into the causes of wilt of cacao it was found from measurements of a large number of fruits that, up to a certain critical size, they were liable to wilt, but beyond that size they usually survived unless attacked by pathogenic organisms. The object of the present investigation was to correlate the findings in the field with the changes in composition occurring in the fruit at different stages of development. A study of the carbohydrate metabolism constituted the first line of attack on the problem. The sampling was extended to include fruits in all stages of development from the time of fertilization until maturity, in order to obtain data for a broad ontogenetic survey of the changes taking place, and also to provide a basis for future biochemical work on the various components of the cacao seed. Hitherto, chemical and biochemical investigations of cacao have chiefly concerned the ripe seed, either fresh or fermented, because this material is more readily available, and is the immediate source of commercial cocoa. There seems little doubt, however, that eventually developmental investigations of various constituents of the cacao seed will have to be undertaken in order to differentiate more clearly the variations, differences in

quality, &c., which are found by manufacturers to exist in different samples of cacao, both in the same and in different countries.

The cacao fruit may be described as a berry, although it is not a typical example. It consists of five fused carpels and contains numerous seeds. During development a white tissue is produced between the seeds and the wall which breaks down on ripening. The mature fruit therefore consists of a thick firm wall enclosing a number of ripe seeds held in the centre of the fruit by vascular tissue which extends from the base to the apex.

II. Methods and Procedure

It was not found possible to use the straightforward sampling technique of tagging fruits at the time that they were set and collecting samples after different periods of time, owing to the fact that as much as 95 per cent. of the fruits are liable to die prematurely. Recourse was made, therefore, to an indirect method. This consisted in employing a size index of development instead of time, and relating this to size-time data already available. Thus, the first size class included fruits between 1·0 cm. and 1·9 cm. in length; the second, 2·0 cm. to 2·9 cm., and so on, up to a size class of 18·0 cm. to 18·9 cm. In this last class two samples were collected, one consisting of unripe fruits and the other of fruits which were just yellowing. Duplicate samples of each class were collected except in the first seven groups, where only sufficient fruits were available to make one whole sample. The samples were collected from a group of 50 budded trees about 20 years old, growing at River Estate, Trinidad. These trees bear long, yellow, furrowed fruits, yielding good Forastero cacao. The average age of the fruits in any class was determined by averaging the growth data obtained in a previous investigation at the same time of the year from a large number of fruits of the same type grown in a neighbouring plot (Humphries, 1942). The age-length relationship adopted is shown in Table I.

The end of November was chosen as a suitable sampling time, because fruits of all sizes were then present on the cacao trees. The fruits were collected, put into tins with tight-fitting lids, and quickly conveyed to the laboratory, where they were weighed and subsampled for the determination of various fractions.

In the following account the sugar values have been expressed as percentages of fresh weight, but where large changes in water content were found, the results have also been expressed on an absolute basis (sugar in pulp), and also on a residual fresh weight (fresh weight *minus* fat), and residual dry weight (dry weight *minus* fat), in order to bring out the changes more clearly, and to determine whether the observed increases or decreases were real or apparent.

Analytical Methods

For the determination of carbohydrates each sample was cut into small pieces and quickly put into boiling alcohol of concentration such that, allowing for the water in the tissue, the final concentration was 80 per cent. Where

necessary, water was added. The sample was then separated from the alcohol, dried in an oven at 100° C., and finely ground in a coffee mill. This powder was then exhaustively extracted with alcohol in a Soxhlet. The two alcohol extracts were finally bulked and made up to volume. Aliquots of the alcohol

TABLE I

Relationship between Age and Length of Cacao Fruits

Length of fruit (cm.).	Average age of fruit (days).
1·0 to 1·9	18
2·0 to 2·9	25
3·0 to 3·9	32
4·0 to 4·9	39
5·0 to 5·9	46
6·0 to 6·9	52
7·0 to 7·9	57
8·0 to 8·9	63
9·0 to 9·9	68
10·0 to 10·9	73
11·0 to 11·9	78
12·0 to 12·9	84
13·0 to 13·9	87
14·0 to 14·9	93
15·0 to 15·9	99
16·0 to 16·9	107
17·0 to 17·9	122
18·0 to 18·9	143
18·0 to 18·9 (ripe)	170

extract were used to determine alcohol-soluble material and soluble carbohydrates. The latter were determined on solutions obtained by evaporating the alcohol *in vacuo*, clearing with basic lead acetate, and precipitating the excess of lead with sodium phosphate. The analytical methods followed were the same as those described by Barnell (1936, 1940). Starch[1] and ether-soluble material were determined on the alcohol-insoluble fraction.

III. FRESH WEIGHT, ABSOLUTE AND PERCENTAGE DRY WEIGHT, AND WATER CONTENT OF THE WALL AND PULP

Each of the samples collected was divided into wall and pulp. This subdivision was easily effected by cutting each fruit into quarters longitudinally and prising out the pulp with the point of a knife. The line of separation was well demarcated in all except the very youngest stage, which was sampled as a whole and counted as wall. In the subsequent discussion the term 'pulp', unless otherwise stated, includes all the tissues inside the wall, and as development proceeds it includes an increasing amount of seed. The fresh weight and dry weight of each sample were determined, and the results obtained are presented in Table II. Each of the two parts of the fruit showed a rapid increase in weight as development proceeded, the wall maintaining its superior

[1] Throughout this paper the term 'starch' implies the substances hydrolysed by means o f undiluted taka-diastase supplied by Parke Davis & Co., Walkerville, Ontario, Canada.

weight throughout development. The ratio of wall to pulp as regards both fresh and dry weight, however, declined progressively until the fruit began to ripen, when the ratio of the fresh weights increased slightly (Table II). The pulp, although decreasing in fresh weight on ripening, increased markedly in

TABLE II

Average Values for Fresh Weight and Dry Weight of Wall and Pulp of a Cacao Fruit during Development

Age of fruit (days).	Average fresh weight of fruit (g.).	Average fresh weight of wall of fruit (g.).	Average fresh weight of pulp of fruit (g.).	Average dry weight of wall of fruit (g.).	Average dry weight of pulp of fruit (g.).	Ratio of fresh weights of wall and pulp.	Ratio of dry weights of wall and pulp.
18	0·183	(0·183)	—	0·0369	—	—	—
25	1·04	0·935	0·105	0·188	0·0142	9·0	13·2
32	2·30	2·128	0·172	0·434	0·0222	12·4	19·5
39	4·35	4·014	0·336	0·698	0·0404	11·9	17·3
46	8·11	7·334	0·796	1·26	0·0953	9·2	13·2
52	13·5	12·17	1·33	2·04	0·157	9·2	13·0
57	21·9	19·32	2·58	3·47	0·255	7·5	13·6
63	29·2	25·84	3·36	4·36	0·369	7·7	11·8
68	41·1	35·40	5·70	5·70	0·569	6·2	10·0
73	54·7	46·37	8·33	6·75	0·768	5·6	8·8
78	89·3	71·63	17·68	9·32	1·70	4·1	5·5
84	115·1	90·84	24·26	11·92	2·23	3·7	5·3
87	156·1	117·65	38·45	15·69	3·64	3·1	4·3
93	199·7	144·12	55·58	19·28	6·45	2·6	3·0
99	260·0	183·40	76·60	24·23	10·57	2·4	2·3
107	346·0	248·60	97·40	34·50	18·94	2·6	1·8
122	423·0	301·10	121·90	42·90	23·91	2·5	1·8
143	478·4	356·00	122·40	49·15	26·71	2·9	1·8
170	496·3	378·50	117·80	50·66	43·25	3·2	1·2

dry weight, and a consequent big decrease in water content was shown. In contrast, the wall showed a very slight *increase* in water content. The absolute amount of water per fruit in different stages of development showed that, during the ripening period, there was no net increase in the amount of water present in the fruit, but that there was a transfer of water from the pulp to the wall. Thus, at 122 days, and before ripening had commenced, the wall of the fruit contained 258 gm. of water and the pulp 98 gm. At 143 days, when the fruit had attained its full size and ripening processes were beginning, the water content of the wall was about 307 gm. and that of the pulp 96 gm., making the total water content of the fruit 403 gm. At the ripe stage (170 days) the absolute water content of the wall was 328 gm., whereas that of the pulp was 75 gm., a decrease of 21 gm., which was exactly balanced by the increase in absolute water content of the wall. This is an opposite effect to that observed in the banana fruit, where water is withdrawn by the pulp from the skin in fruits ripening off the plant (Barnell, 1941).

   The changes in percentage water content of both the wall and the pulp are shown in Figs. 1 A and 1 B, the data being calculated from Table II. The
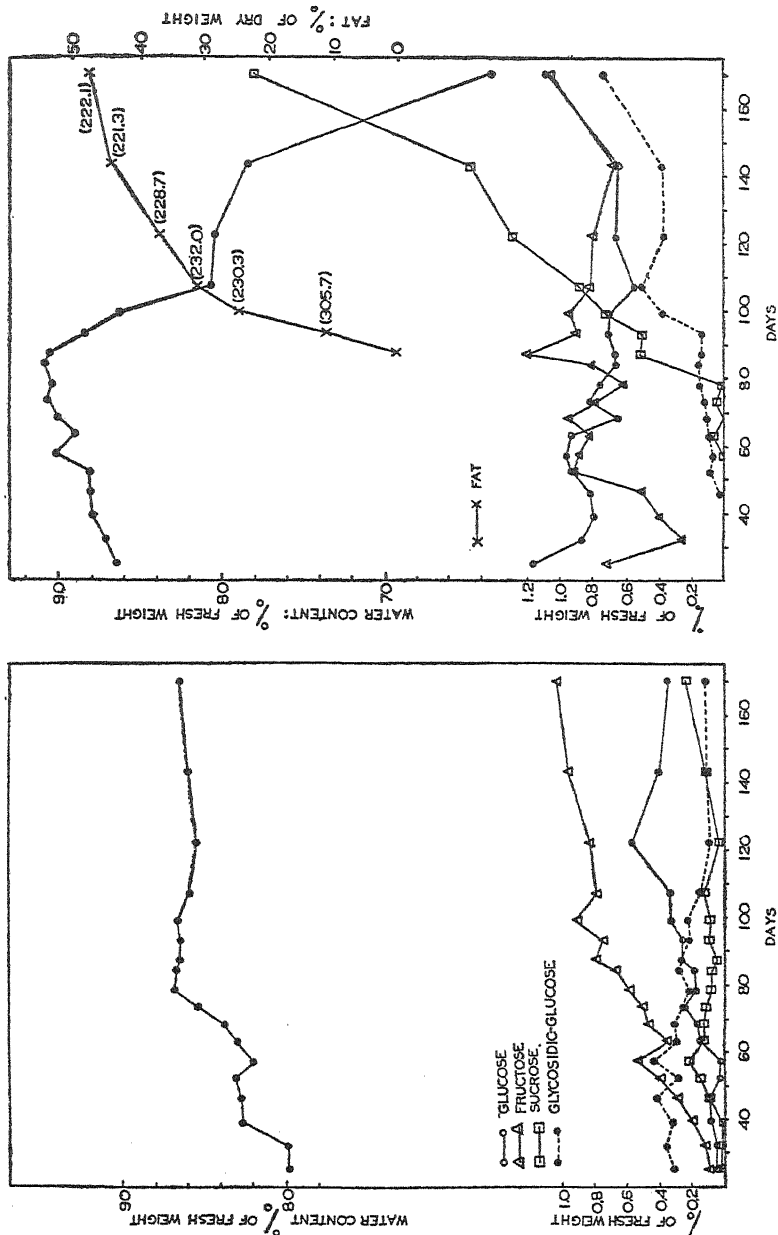
FIG. 1 B.

FIG. 1 A.

FIG. 1 A. The changes in water content and amounts of glucose, fructose, sucrose, and glycosidic-glucose, expressed as percentage of the fresh weight, in the wall during development.   FIG. 1 B. The changes in water content and amounts of glucose, fructose, sucrose, glycosidic-glucose, expressed as percentage of the fresh weight, and of fat expressed as percentage of the dry weight, in the pulp during development. The figure in brackets opposite any particular fat value indicates the saponification value of the fat at that stage.

E

percentage water content of the wall showed a progressive increase up to about 75 days, after which there was a slight downward drift and then a slight increase again at maturity. There was also a progressive increase in the percentage water content of the pulp up to about 70 days, when it remained approximately constant until 85 days, after which there was a decrease until 122 days, and then a less marked decrease until maturity. It will be shown subsequently that the rapid decrease in percentage water content corresponded to the time when rapid fat formation occurred in the seed. Both the wall and the pulp therefore reached their maximum percentage water content at about 75 days. It was previously found that this age appeared to be a critical one in the development of the fruit. Humphries (loc. cit.) found from field observations that physiological wilt of cacao fruits may occur at any age up to about 70 days (fruits approximately 10 cm. length), after which wilting from purely physiological causes rarely occurs. It appears, from data which will be presented subsequently, that certain important metabolic changes begin to occur, especially in the pulp, at the time when the percentage water contents of the wall and pulp reach their maxima. These changes coincide with the rapid development of the embryo and the accumulation of food reserves in the seed. After these metabolic changes have been initiated, the fruit does not appear to be susceptible to physiological wilt. An outward manifestation of these metabolic changes is evident in the change of the ratio of fruit length to fruit diameter which occurs at the critical period (about 75 days). The change in ratio of fruit diameter to fruit length is shown in Fig. 2. This curve is constructed from a large number of measurements of fruits of the same variety as used throughout this work, in all stages of their development. The ratio remains practically constant until about 70 days, and then begins to increase, i.e. the fruit commences to swell. The increases in dry weight of both wall and pulp are exponential in the early stages of development, the logarithms of the dry weights plotted against time giving good approximations to straight lines (Fig. 3). Values for the dry weight of pulp, calculated from the equation of best fit obtained by the least squares method, give good agreement with the experimental values up to at least 70 days. In the case of the wall data, however, the points lie about two straight lines with a well-marked point of inflexion (Fig. 3). The change in the rate of accretion of dry matter by the wall occurs at about 50 days, at which age Cheesman (1927) found the first division of the fertilized ovum to take place. The effect of the initiation of embryo development appears to cause a decrease in the rate of accumulation of dry matter in the wall; this suggests that a redistribution of translocation products occurs, with the result that the wall receives a smaller proportion of nutrient than previously. The particular age at which this happens is also characterized by certain definite changes in carbohydrate metabolism (see p. 10).

The initial rate of accumulation of dry matter in the pulp is maintained over a much longer period than in the case of the wall. The equations of best fit for the dry weight data of the wall and pulp are given in Table III. The interest-

ing fact emerges that the relative growth-rates of the wall and pulp (as measured by accumulation of dry matter) are practically the same up to 50 days, that is, before development of the embryo commences.
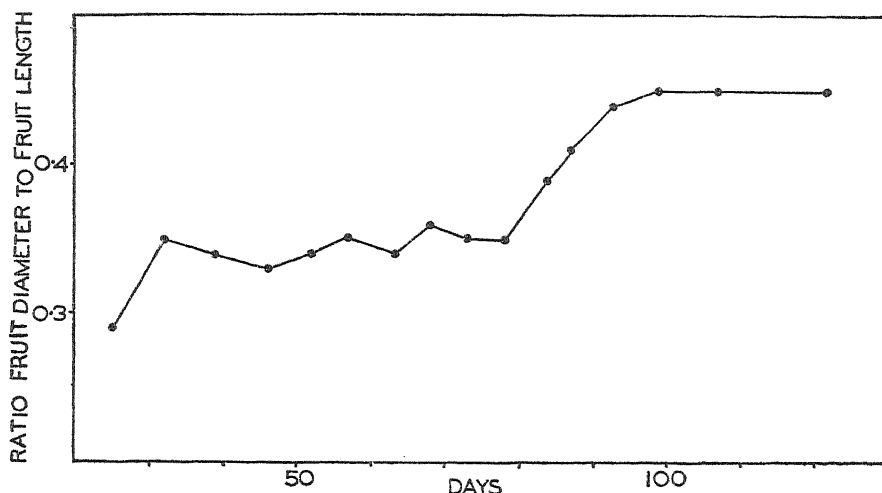
FIG. 2. The changes in the ratio of fruit diameter to fruit length during development.

TABLE III

Equations of Best Fit for Changes in the Logarithm of Dry Weight with Time of Wall and Pulp for the Ages shown

Dry-weight wall up to age 52 days: $y = -1.6335 + 0.0379x$
Dry-weight wall age 57 to 87 days: $y = -0.6554 + 0.0207x$
Dry-weight pulp up to age 70 days: $y = -2.7702 + 0.0372x$

IV. ALCOHOL-SOLUBLE MATTER OF THE WALL AND PULP

The presence in plant material of an alcohol-soluble fraction other than carbohydrate has recently been discussed by Barnell (1936, 1938, 1940) and Archbold (1938). Data are provided for this fraction in the development of the banana fruit and of wheat and barley plants, and its importance as an indicator of possible trends in the metabolism of substances other than sugars during development is stressed. The suggestion that this fraction needs further investigation is well supported by data obtained in the present investigation (Fig. 4). In the case of the wall, the content of sugars, expressed as a percentage of the alcohol-soluble material, showed a steady and consistent increase until about 100 days, reaching a maximum of nearly 40 per cent. During the rest of the developmental period the percentage of sugars remained practically constant. In the pulp, however, the changes in the amount of sugars present in the alcohol-soluble material at different times were clearly

marked. In the early stages the percentage of sugar dropped at first and then rose to a maximum at approximately 70 days, a point which has already been shown to be critical in the development of the pod. Subsequently, there was
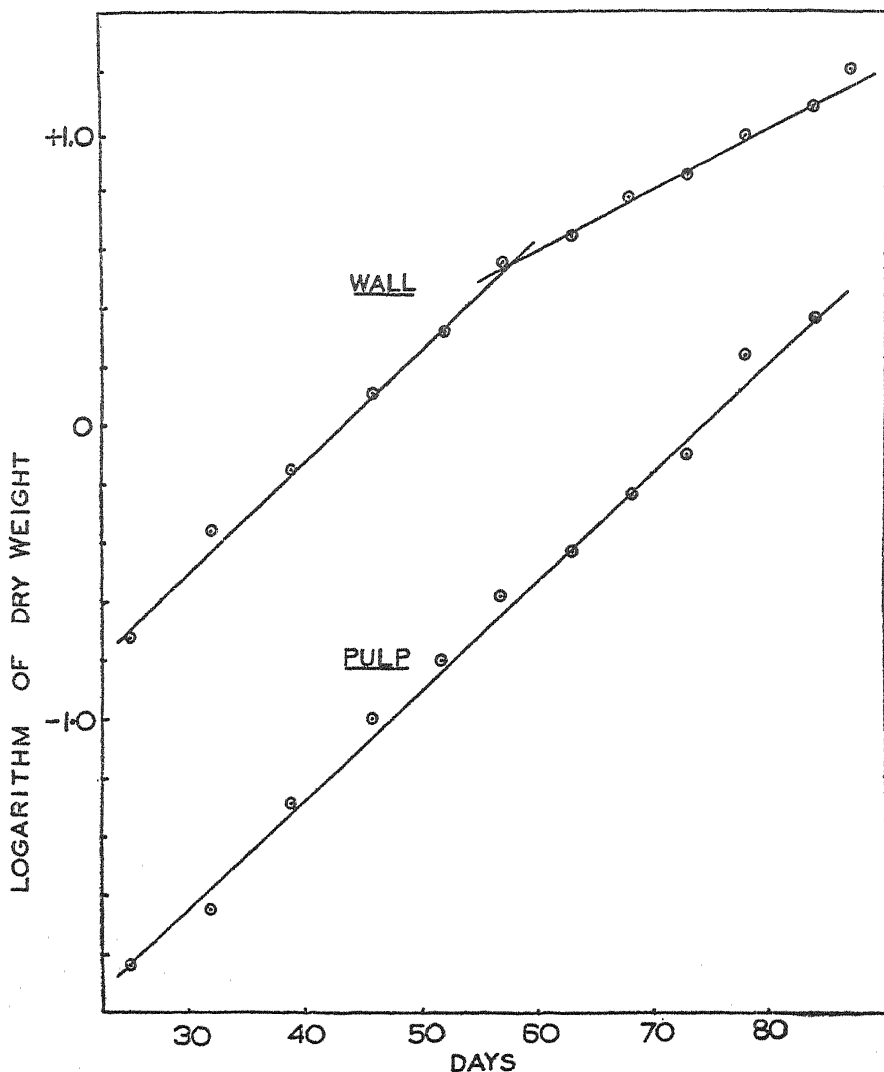
FIG. 3. The lines of best fit for the logarithmic increase in dry weight of wall and pulp. Note the inflexion in the wall curve between 50 and 60 days.

a drop and then a sharp increase to a maximum at about 90 days, fructose accounting for about half of the total sugars present. At this point the three sugars, sucrose, glucose, and fructose, made up just over half of the alcohol-soluble material, and fat was also beginning to form in the seed. The period between 70 and 90 days, where non-sugars were considerably in excess of

sugars in the alcohol-soluble fraction, probably marked the initiation of chemical changes leading to the formation of precursors of the seed fat. There is need for further information on this point. The non-sugars fraction decreased during the next 20 days, and then increased steadily until maturity. Thus the drift of the non-sugars in the pulp is in marked contrast with that

TABLE IV

Alcohol-soluble Material and Carbohydrates in the Wall at Different Stages of Development

Age of fruit (days).	Alcohol-soluble material per cent. fresh weight.	Glucose per cent. fresh weight.	Fructose per cent. fresh weight.	Sucrose per cent. fresh weight.	Glycosidic-glucose per cent. fresh weight.	Taka-diastase hydrolysable products per cent. dry weight.
25	3·54	0·06	0·09	0·02	0·31	0·25
32	3·75	0·05	0·12	0·01	0·36	0·29
39	4·00	0·09	0·20	0·00	0·32	0·38
46	3·77	0·10	0·29	0·10	0·42	0·27
52	3·70	0·03	0·40	0·15	0·29	0·42
57	3·52	0·02	0·53	0·22	0·44	0·60
63	3·74	0·15	0·35	0·14	0·30	0·37
68	3·29	0·17	0·47	0·13	0·31	0·47
73	3·48	0·25	0·50	0·12	0·26	0·45
78	3·32	0·18	0·59	0·09	0·22	0·51
84	3·92	0·19	0·67	0·08	0·29	0·49
87	4·04	0·27	0·80	0·05	0·27	0·51
93	3·72	0·26	0·75	0·10	0·22	0·49
99	3·48	0·33	0·92	0·09	0·23	0·72
107	3·76	0·34	0·79	0·13	0·15	0·53
122	3·91	0·57	0·83	0·04	0·10	0·36
143	4·03	0·41	0·97	0·12	0·12	—
170	4·22	0·35	1·03	0·24	0·12	0·41

of the non-sugars in the wall, not only in the more regular trend in the latter, but also in the fact that a greater proportion of the alcohol-soluble material of the pulp is present as sugars. Mucilage is a noteworthy component of the alcohol-insoluble substances in the wall, but no satisfactory method has so far been found for its estimation.

V. CARBOHYDRATES OF THE FRUIT WALL

(a) Sucrose, glucose, and fructose

The contents of sucrose, glucose, and fructose expressed as percentages of the fresh weight, throughout the development of the fruit wall, are shown in Fig. 1 A, constructed from data in Table IV. In the early stages fructose was the sugar present in greatest amount and it showed an opposite drift from glucose. The fructose content increased quite rapidly at first from about 0·1 per cent. at 25 days to about 0·5 per cent. at 57 days. Subsequently, its percentage amount fell somewhat and then increased steadily to 100 days, when

there was a small decrease and then an increase to maturity. The quantity of glucose was about half that of fructose in the early stages, and after a slight rise it decreased to a minimum value at 57 days, a time when the percentage of fructose had reached its first maximum. This age corresponds to the period when a change in the growth-rate of the fruit wall occurs, and cell division of
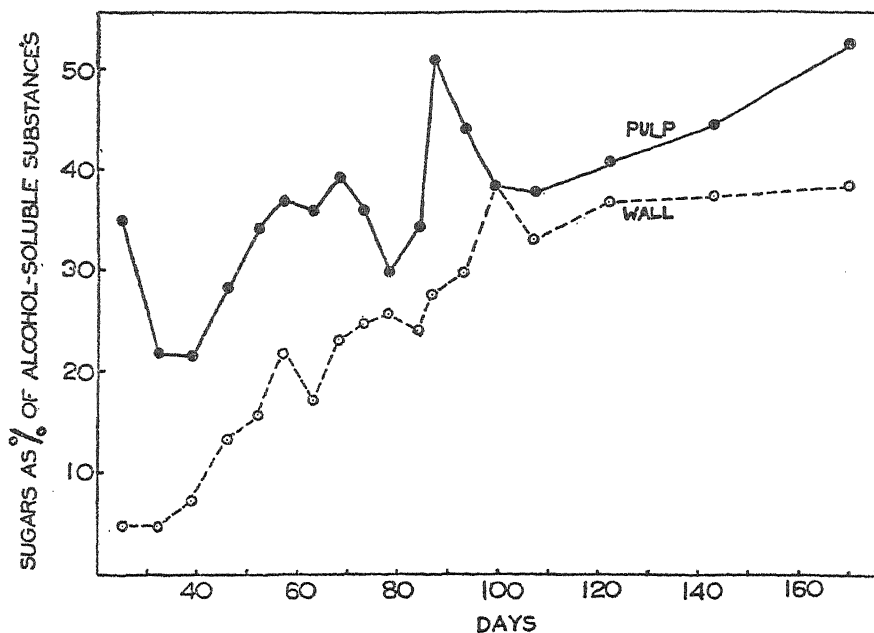
FIG. 4. The sugars (glucose, fructose, and sucrose), of the wall and pulp, expressed as percentage of the alcohol-soluble material at various times during development.

the embryo is initiated. From this point onwards the amount of glucose showed a steady increase, reaching a maximum around 120 days, after which there was a slight decline, probably associated with changes taking place in the wall of the ripening fruit. Sucrose was present in very small quantities until 40 days, but its amount subsequently increased rapidly to a maximum at 57 days. Afterwards there was a decline until about 90 days and then a slight increase to a fairly constant but fluctuating value which was maintained until ripening commenced, after which the value again increased, reaching the highest value of the whole period of development.

(b) *Glycosidic-glucose*

The percentage amount of glycosidic-glucose remained fairly constant at first, though subject to fluctuation. After approximately 70 days its amount began to fall progressively until the time when the fruit began to ripen, after which it remained constant. Knapp and Hearne (1939) have pointed out that leuco bodies are present in various tissues of ripe Criollo cacao fruits, in-

cluding the wall, in the form of glycosides. There is no reason to suppose that
Criollo and Forastero cacao fruits are different in this respect.

(c) Starch

The amount of substance hydrolysable with taka-diastase in the fruit wall
was small throughout development (see Fig. 5 and Table IV), and was subject
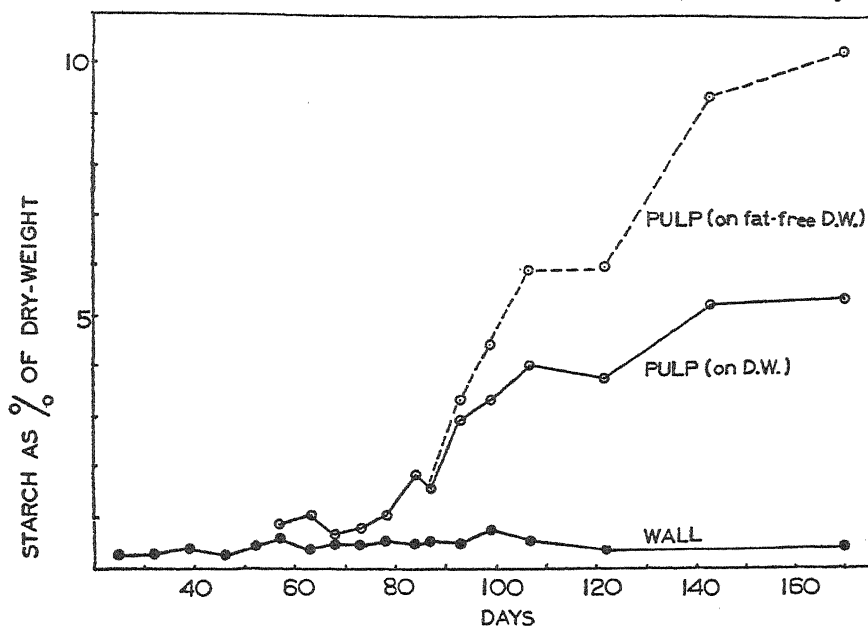
FIG. 5. Taka-diastase hydrolysable products of the wall and pulp at various times during
development. The data for the pulp are expressed on both dry weight and fat-free dry weight.

to very little variation. True starch was detected by microscopical examina-
tion only in the epidermal hairs, and this appeared to be of a transient nature.

VI. CARBOHYDRATES OF THE PULP

(a) Source, glucose, and fructose

The percentages of both glucose and fructose were greater in the initial
stages in the pulp than in the wall, and, unlike the wall, glucose was present in
greater amount at first (see Fig. 1 B and Table V). The percentage value for
glucose content in the earliest stage examined (i.e. 25 days) was the highest
recorded. There was a decrease from this value until 39 days, and then a rise
to a maximum at nearly 57 days, a period when active cell division is initiated
in the embryo. From this time onwards there was a downward drift in
glucose content until the fruit began to turn yellow (i.e. 143 days), when the
value increased again. Fructose was present in smaller amount than glucose
in the initial stages, and, like glucose, showed a decrease at first but rapidly

increased again and became equal to the glucose content at about 50 days. The fructose content then remained approximately equal to the glucose content until about 80 days, when there was a marked rise in the amount of fructose to a maximum at 87 days, a time when fat formation in the seed was initiated.

TABLE V

Alcohol-soluble Material, Carbohydrates, and Fat in the Pulp at Different Stages of Development

Age of fruit (days).	Alcohol-soluble material per cent. fresh weight.	Glucose per cent. fresh weight.	Fructose per cent. fresh weight.	Sucrose per cent. fresh weight.	Glycosidic-glucose per cent. fresh weight.	Taka-diastase hydro-lysable products per cent. dry weight.	Ether-soluble material per cent. dry weight.
25	5·39	1·17	0·71	0·00	0·00	—	—
32	5·17	0·87	0·25	0·00	0·00	—	—
39	5·62	0·80	0·40	0·00	0·00	—	—
46	4·67	0·82	0·50	0·00	0·03	—	—
52	5·46	0·94	0·92	0·00	0·09	—	—
57	5·05	0·97	0·89	0·00	0·07	0·88	—
63	5·11	0·94	0·82	0·07	0·09	1·05	—
68	4·00	0·65	0·96	0·00	0·11	0·68	—
73	4·85	0·82	0·78	0·05	0·12	0·77	—
78	4·60	0·76	0·61	0·01	0·15	1·05	—
84	4·29	0·66	0·80	0·00	0·16	1·82	—
87	4·61	0·67	1·21	0·51	0·14	1·59	0·45
93	4·77	0·71	0·89	0·50	0·14	2·94	11·17
99	6·13	0·70	0·95	0·71	0·38	3·34	24·67
107	6·09	0·55	0·82	0·88	0·50	4·04	31·49
122	6·79	0·66	0·80	1·30	0·37	3·77	36·95
143	6·46	0·64	0·66	1·56	0·38	5·22	44·31
170	9·68	1·09	1·06	2·90	0·73	5·34	47·64

Subsequently, the amount declined until 143 days, when it again became equal to the amount of glucose, and then increased equally with glucose content. Sucrose appeared to be absent from the pulp during the first 80 days, small quantities only being recorded for the 63- and 73-day samples. In the 87-day samples, however, sucrose content rose to 0·5 per cent., and continued to rise during the rest of the development, reaching nearly 3 per cent. in the ripe pulp. The onset of sucrose formation was coincident with the beginning of fat formation in the pulp.

At the time of fat formation in the pulp a large decrease in the water content of the tissue took place, so that, in order to follow more exactly the changes in fructose and glucose contents during this period, the values for these sugars have been expressed as percentages of the fat-free fresh weight and fat-free dry weight, as well as on an absolute basis, per pulp (Table VI). The fructose values which showed a decrease when expressed as percentages of the whole pulp also showed a decline when expressed on either fat-free fresh weight or fat-free dry weight, so that it is assumed that the decrease in amount of fructose is real. On the absolute basis, it appears that the amount of fructose in the

pulp reached a steady value after about 99 days until the onset of ripening, with the exception of a high value at 122 days. In the case of glucose, it appears that its percentage amount reckoned on fat-free fresh weight remained fairly constant until the onset of maturity, when it again increased. On the fat-free dry-weight basis, there was a slight decline until 107 days, after which it remained constant. On the absolute basis, there was a steady increase throughout the period.

TABLE VI

Changes in the Fructose and Glucose Values of the Pulp, from the Onset of Fat Formation until Maturity, expressed on Fat-free Fresh Weight, Fat-free Dry Weight, and on Absolute Basis

	Fructose.			Glucose.		
Age of pod (days).	Per cent. fat-free fresh weight.	Per cent. fat-free dry weight.	Per fruit ('pulp'). (g.)	Per cent. fat-free fresh weight.	Per cent. fat-free dry weight.	Per fruit ('pulp'). (g.)
87	1·21	12·69	0·46	0·67	7·11	0·26
93	0·90	8·86	0·49	0·72	6·68	0·40
99	0·98	9·16	0·73	0·72	6·70	0·54
107	0·87	6·16	0·79	0·59	5·50	0·53
122	0·86	6·46	0·97	0·71	5·42	0·80
143	0·73	5·39	0·79	0·71	5·47	0·79
170	1·29	5·50	1·24	1·32	5·63	1·28

From the data available no definite conclusions can be reached regarding the chief sugar concerned in fat synthesis in the cacao seed. This is because the pulp was sampled as a whole, and it is not possible to identify the changes occurring separately in the white tissue surrounding the testa, the testa itself, and the kernel of the seed, all of which together in the present paper have been designated 'pulp'. It is particularly difficult to make a partition in the early stages of fat formation when the seed has liquid contents. To obtain some indication of the changes in the kernel alone of the seed, a few samples were collected of ripe and almost ripe fruits in which the desired partition was more easily effected. These samples were collected in May towards the end of the dry season and about 6 months later than the samples of the main experiment. These 'out of season' fruits had a different age-size relationship from the fruits collected in November; hence it was only possible to fix their ages approximately, but they were estimated to lie between 143 and 170 days, i.e. when the fruit is in the ripening stage. The data of Table VII show that, in these extra samples, the glucose and fructose concentrations were large in the white tissue surrounding the seed and comparatively small in the kernel, whereas the sucrose concentrations were approximately the same in both white tissue and kernel, except in the last sample (No. 4) which was obtained from the almost ripe fruits, where the percentage of sucrose was much greater in the white tissue than in the kernel. The three sugars, glucose, fructose, and sucrose, together accounted for over 70 per cent. of the alcohol-soluble material

of the white tissue, but in the kernel these sugars only contributed about 30 per cent. of this fraction. The glycosidic-glucose was greater in the kernel than in the white tissue in all cases.

TABLE VII

Partition of Pulp into Kernel and White Tissue + Testa, from Nearly Ripe Fruits

Kernel of bean.

Sample No.	Dry matter per cent. fresh weight.	Alcohol-soluble material per cent. fresh weight.	Glucose per cent. fresh weight.	Fructose per cent. fresh weight.	Sucrose per cent. fresh weight.	Glycosidic-glucose per cent. fresh weight.	Taka-diastase hydrolysable products per cent. dry weight.
1	37·98	8·95	0·11	0·11	2·76	0·67	6·85
2	41·88	8·01	0·20	0·15	3·50	0·68	7·86
3	34·97	8·31	0·18	0·11	2·36	0·55	7·36
4	53·32	8·11	0·27	0·06	1·20	0·61	—

White tissue + testa.

1	14·68	8·79	1·60	1·58	2·32	0·12	1·07
2	17·21	8·24	1·93	1·53	3·43	0·12	1·26
3	14·40	7·40	1·29	1·77	2·17	0·18	0·74
4	14·97	—	1·48	1·64	4·19	0·21	—

The sugars present in the cacao seed have been variously reported, and Knapp (1937) has pointed out that the sugars occurring in the white tissue and kernels are generally returned as sucrose and reducing sugars. Brill (1915) reported the occurrence of an enzyme, raffinase, both in the pulp and kernels of the cacao seed. There is a possibility, therefore, that the sugar, raffinose, is present. This, like sucrose, yields reducing sugars by the action of invertase. It was confirmed, however, that equal quantitites of fructose and glucose were obtained by the hydrolysis of the sugar, so that it would not appear that raffinose is present in any appreciable amount.

(b) Glycosidic-glucose

This was low in amount at first, increasing very slowly until about 93 days, after which it increased rapidly until about 107 days, when it remained fairly constant except for a sharp increase on ripening. The period of rapid increase in the percentage amount of glycosides coincided with the onset of fat formation and the time when the cotyledons began to develop a purple colour. Lawrence, Price, Robinson, and Robinson (1938) have shown that this colouring matter is an anthocyanin, namely, cyanidin-3-monoside. The sequence of events in the development of glycosidic-glucose in the wall and in the pulp followed opposite trends, being high in the wall at first and subsequently decreasing, and vice versa in the pulp.

(c) *Starch*

In the pulp, starch content, expressed as a percentage of dry weight, remained low until about 80 days, when there was a rapid rise up to 100 days, after which the rate of increase became less but the increase continued until maturity (see Fig. 5). Probably a more exact picture of the drift of the taka-diastase hydrolysable products might be obtained by calculating the results on a fat-free basis, since, after about 90 days, an increasing amount of the dry weight was fat, until at maturity nearly 50 per cent. of the dry weight was fatty material. These figures, expressed on the fat-free dry weight, are also shown in Fig. 5. It is seen that the rise in amount of starch was rapid from 90 days onwards, the rate of increase being maintained almost until maturity, when about 10 per cent. of the non-fatty material of the pulp consisted of substances hydrolysed by taka-diastase. At maturity, most of this substance occurred in the kernel of the seed (see Table VII).

VII. Development of Fat in the Pulp

It was comparatively easy to estimate the fat present at different stages by means of ether extraction of the alcohol-insoluble residue. Although cacao fat is slightly soluble in hot alcohol, it separates out on standing at room temperature. It was found that fat formation commenced at a definite stage and then fat accumulated rapidly. Reference to Fig. 1 B shows that at 87 days (about 13 cm. length) the ether-soluble material, expressed on original dry-weight basis, was less than 1 per cent., but at the next stage sampled, estimated to be 93 days old, the fat content had increased to 11 per cent. This rapid rate of increase was continued for approximately 6 days, when the fat content had reached nearly 25 per cent. of the dry weight of the pulp. Subsequently, the increase in the amount of fat was not so rapid but continued until the time that the fruit ripened. The saponification value of the fat was high in the initial stages (305·7 at 93 days), but decreased rapidly up to 100 days, after which there was a gradual decrease with time (see Fig. 1 B). The seeds had liquid contents during the period of rapid decrease in saponification value. At about 100 days the seeds had solid contents, having passed through an intermediate gelatinous stage. In the stages immediately before ripening, fat was present in small amount in the testa, but absent in the ripe seeds. The testa fat was yellowish in colour and appeared to be different from the fat extracted from the kernels.

VIII. Discussion

The data presented indicate that the growth cycle of the cacao fruit, which is approximately 170 days, falls naturally into two phases. The first of these phases, occupying about 75 days, is essentially a period of development during which the percentage water contents of both wall and pulp increase from the time of fertilization and reach their maxima at the end of this phase. It was previously found by observation of developing fruits that they are susceptible to physiological wilt at any age within this phase. The first phase may be divided into two periods. The first of these covers an interval of about 50

days, at the end of which the first division of the zygote occurs. Fertilization is followed by rapid exponential increase in dry matter of both wall and pulp, the constant up to 50 days being the same in both cases. The percentage amount of fructose in the wall increases throughout this period, while glucose increases slightly and falls to a minimum at the time of the division of the zygote. Sucrose in the wall reaches a maximum at the end of this period. In the pulp the percentages of both fructose and glucose fall and then rise again to approximate equality at the end of the period.

The second period (50–75 days) of the first phase commences with the division of the zygote, and the metabolic changes are undoubtedly associated with the preliminary development of the embryo. This second period is not well defined as far as the carbohydrate changes in the wall are concerned, but it is a time when the percentage water content is still increasing. The rate of accumulation of dry matter in the wall falls to a lower but constant value in this period while the rate in the pulp is maintained. During this period the concentrations of fructose and glucose are practically equal in the pulp and show a downward trend. The amount of sucrose is negligible.

The second phase may be described as one of active metabolism and falls into three periods. The first period (75–87 days) is not reflected by the changes in the wall where the drifts established at the division of the zygote continue. In the pulp, however, rapid changes are apparent; active sucrose formation commences, while fructose concentration rises to a maximum at the end of the period. Glucose content continues its downward trend. In the middle of the period the ratio of soluble sugars to alcohol-soluble material is low. It is of interest to speculate as to whether, during this period, reducing sugars are utilized to form the precursors of the seed fat. It is possible that the process of fat formation commences in the previous period when the concentrations of glucose and fructose are equal. This raises the possibility that sucrose is the starting-point for certain of the precursors of the fat, but that it is so actively metabolized that it does not accumulate. Without additional evidence, further speculation would be unprofitable at this stage.

The second period (87–143 days) of the second phase is dominated by the rapid accumulation of fat in the pulp. This fat has a high saponification value at first which rapidly decreases. There is a marked fall in the concentration of fructose throughout the period, while the glucose concentration remains practically constant. Sucrose and starch continue to accumulate at a rapid rate. Glycosides increase rapidly soon after the beginning of this period at the same time that the cotyledons assume a purple colour. Fat is present both in the testa and in the cotyledons of the seed at this stage.

In the third period of the second phase (143–170 days), ripening takes place. There is no further increase in the size of the fruit. In the wall, fructose continues to increase and glucose to decrease. Sucrose shows a slight increase. In the pulp, sucrose, starch, and fat continue to increase, and the concentrations of glucose and fructose again become equal. No fat is present in the testa at maturity. Anthocyanin continues to increase in amount. At the

end of this period the fruit is ripe, as shown externally by colour changes and internally by the separation from the wall of the seeds, each surrounded by white tissue.

This account is a brief survey only of the changes in water content, dry matter, carbohydrates, glycosides, and fat of the wall and pulp of the cacao fruit during development, and is intended to form a starting-point for more detailed investigations of the numerous metabolic changes which have been outlined. It would be desirable, for instance, as Hilditch (1940, p. 269) has pointed out, to obtain further evidence concerning the changes leading to the formation of stearic, oleic, and palmitic acids, which are the chief fatty acids present in the cacao seed. Interesting lines for future investigation are the relation of the metabolic changes outlined in this paper with nitrogen metabolism during development, leading to the formation of such substances as catechin (Adam, Hardy, and Nierenstein (1931)), anthocyanin (Robinson *et al.* (1938)), caffeine, and theobromine.

IX. SUMMARY

1. The changes in water content, dry matter, carbohydrate, and glycosidic-glucose in the wall and pulp of the cacao fruit during development have been followed from fertilization to maturity.

2. It is shown that the growth period of the fruit may be divided into two phases. The first is a developmental phase occupying about 75 days, during which period the fruit is susceptible to physiological wilt. The second is a period of active metabolism, during which fat, starch, and sucrose accumulate in the seed.

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Studies in the Comparative Morphology of the Algae

III. Evolutionary Tendencies and Affinities among Phaeophyceae

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With four Figures and one Diagram in the Text

PHAEOPHYCEAE and Rhodophyceae alone among the Algae show a marked advance in somatic organization, which is associated with considerable reproductive specialization. It is therefore to these two classes that we must for the most part look for examples of evolutionary trends that may be of significance in relation to the origin of archegoniate plants. The majority of Rhodophyceae have evolved in directions that distinguish them sharply from other Algae and, both in their customary pseudo-parenchymatous (filamentous) construction and in the many peculiar features presented by their reproductive processes, have clearly followed lines that exhibit few points of contact with the direction of evolution of land plants. Although they afford some interesting side-lights on evolutionary tendencies among Algae, they do not contribute materially towards an understanding of the mode of origin of bulkier types of plant-body. The Phaeophyceae, on the other hand, with a much wider range in somatic structure and a much greater plasticity in methods of reproduction, provide copious material for a study of evolutionary advance. This fact was recognized by Church (1920) in his memoir on 'The Somatic Organization of the Phaeophyceae', although his argument was obscured by the stressing of many non-essential points, as well as by a number of unfounded assumptions. It is the fact that the Phaeophyceae alone among Algae have produced a diversity of massive plant-bodies with a *true parenchymatous structure* that makes them especially valuable for a study of evolutionary tendencies among non-vascular plants.

The Evolutionary Tendencies recognizable among Ectocarpales

The assemblage of varied genera (with the omission of the oogamous Sporochnales and Desmarestiales), which can, with Oltmanns (1922), be classed as Ectocarpales, shows on a truly magnificent scale a variety of attempts in the direction of evolution of an elaborate sporophyte in what are no doubt relatively primitive Phaeophyceae. Their primitive status is indicated by the universal heterotrichy in the early stages of development (Fritsch 1942a, p. 402), as well as by the prevalent isogamy. The interrelations

of the many genera included in this order are difficult to elucidate, but there can probably be no doubt that Ectocarpus-like forms represent the starting-points of most, if not of all, of the various evolutionary series that are distinguishable (cf. p. 70). The species of Ectocarpus, with their simple heterotrichous construction, isomorphic alternation, and extreme plasticity in methods of reproduction, represent the most primitive forms that are known among present-day Ectocarpales.

From primitive Phaeophyceae of this kind two contrasting types of thallus-structure originated, represented by the haplostichous and polystichous Ectocarpales of Kuckuck (1929). The former have thalli built up from one or more of the erect filaments of the primary heterotrichous stage, the numerous laterals of these filaments being compacted to form a more or less firm pseudo-parenchymatous structure in which the various branches are bound together by mucilage. Such uni- and multi-axial types are not as sharply segregated as among Florideae, since both structures commonly occur in closely allied genera; thus Castagnea is multi- and Mesogloea (Fig. 1, G) uni-axial. The multiaxial haplostichous condition appears on the whole not to have enjoyed any great measure of success among Phaeophyceae, since outside the Ecto-carpales it is realized only, and here in a specialized form, in the Cutleriales. Among Chlorophyceae parallel types occur only among Siphonales (Codi-aceae), although multiaxial structure is indicated in Chaetophora.

The polystichous Ectocarpales, on the other hand, are true parenchymatous types, producing their mature diploid thalli by septation in diverse planes of the erect threads of the juvenile heterotrichous stage (Fig. 1, C, I, M; cf. also Fig. 2, B in Fritsch, 1942a). The bulk of the more specialized Phaeophyceae exhibit this type of structure. Its origin marked a most significant evolution-ary advance, since in it lay the germ for the development of a plant-body of almost unlimited size. Outside the Brown Algae, it is represented only in a few Ulotrichales and Bangiales. The Ulvaceae among Chlorophyceae show an especially striking parallel, although only in vegetative construction, with the polystichous Punctariaceae among Phaeophyceae; Enteromorpha paralleled by Phaeosaccion, Ulva by Punctaria, and Monostroma by Omphalophyllum.

In all polystichous Ectocarpales in which a considerable amount of longi-tudinal septation takes place there is a marked tendency after the early divisions for further segmentation to be restricted largely to the outer cells. The more internal ones are passively distended by the resulting surface-enlargement which operates both in the transverse and longitudinal directions. There thus arises a distinction between a small-celled peripheral cortex (Fig. 1, L, c) and a large-celled medulla (*me*) with elongate cells. The consequent tissue-tensions (Küster, 1899, p. 828; Murbeck, 1900, p. 13)—usually more marked among the higher parenchymatous forms (Wille, 1897, p. 13; Jönsson, 1901, p. 20; Holtz, 1903, p. 25)—may, if the peripheral growth ensues rapidly, lead to the production of a hollow thallus (Asperococcus, Scytosiphon). In diverse instances, as girth increases, the faculty for division becomes largely restricted to the superficial layer of cells, which divide by anti- and periclinal
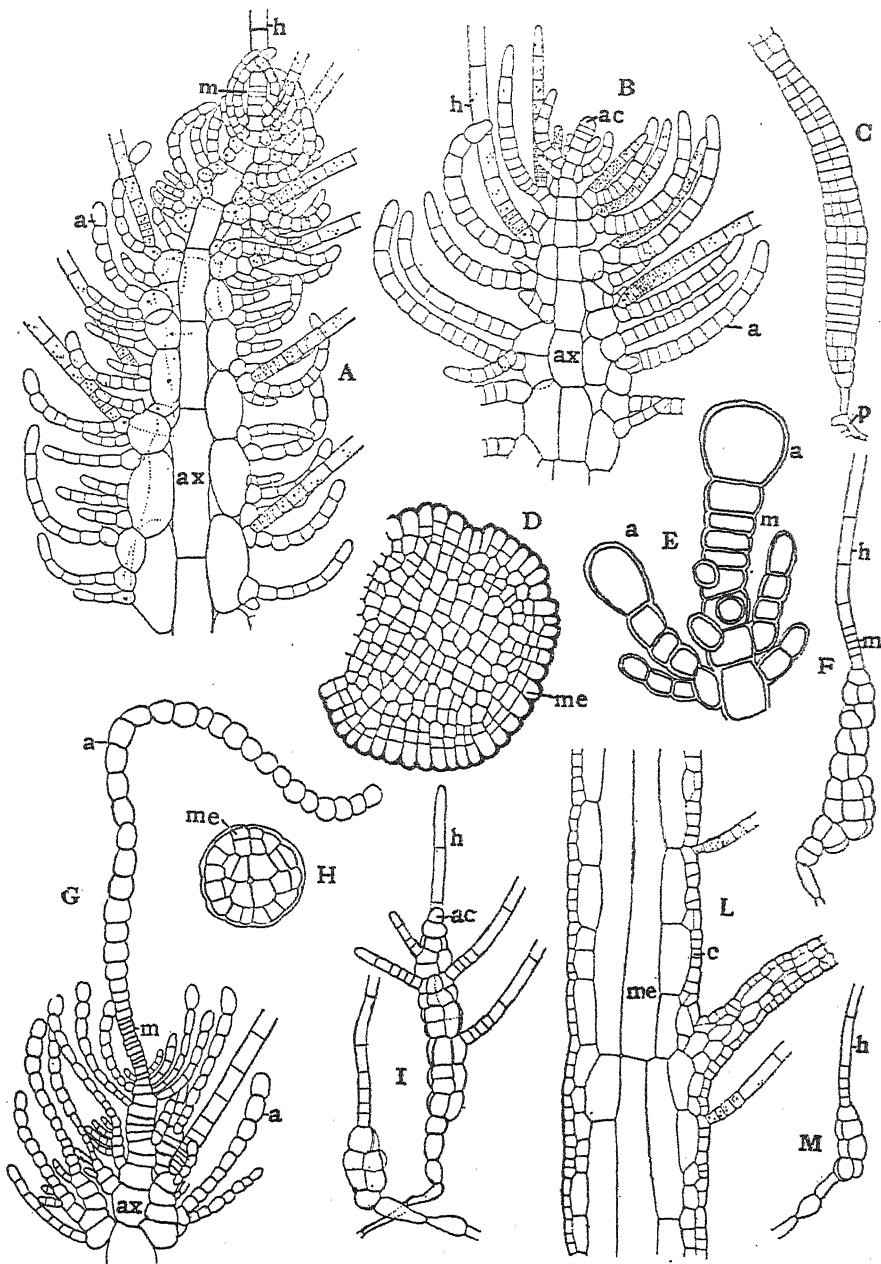
FIG. 1. A, *Acrothrix gracilis* Kyl., apex of plant. B, *Nemacystus flexuosus* (Ag.) Kyl., ditto. C, *Punctaria latifolia* Grev., young plant. D, *Cladostephus verticillatus* Lyngb., transverse section of a lateral branch. E, *Sphaerotrichia divaricata* (Ag.) Kyl., apex of axis. F, I, M, *Dictyosiphon foeniculaceus* (Huds.) Grev., young plants of diverse ages. G, *Mesogloea Leveillei* (J. Ag.) Menegh., apex of plant. H, *Phloeospora brachiata* (Harv.) Born., transverse section. L, *Dictyosiphon foeniculaceus* (Huds.) Grev., longitudinal section of older plant. *a*, assimilator; *ac*, apical cell; *ax*, axial thread; *c*, cortex; *h*, hair; *m*, meristem; *me* (in D and H), meristoderm; *me* (in L), medulla; *p*, prostrate system. (C, D, F, I, M after Sauvageau; E after Kylin; the rest after Kuckuck.)

walls (Fig. 1, H, *me*) and constitute a definite superficial meristem (a *meristo-derm* in the sense of Sauvageau, 1918, p. 99). Such localized meristematic activity is never very pronounced among the polystichous Ectocarpales, but it is marked in certain Sphacelariales (Fig. 1, D, *me*) and attains to a great degree of prominence in Laminariales and Fucales.

A large proportion of the Ectocarpales exhibit intercalary growth. In many species of Ectocarpus, as well as in other Ectocarpaceae, the distribution of cell-division is quite irregular, and it is only in a limited number of them (e.g. *E. irregularis* Kütz.) that definitely localized intercalary meristems occur. Such are usually situated at the base of a colourless hair composed of a limited number of elongate cells, affording the trichothallic growth (Janczewski, 1875, p. 105; Kuckuck, 1895*a*, p. 179) which is characteristic of many Ectocarpales and also typically developed in Desmarestiales and Cutleriales. While the trichothallic meristem in all these instances occupies a subapical position owing to the bulk of the segments being cut off ab-apically, the nature of the overlying cells tends to vary. In Acrothrix (Fig. 1, A), for example, the meristem (*m*) is capped by a colourless hair (*h*), as in the species of Ecto-carpus, whereas in Elachista, Myriogloea, Desmarestiales (Fig. 2, G), and Cut-leriales the surmounting structures are assimilatory hairs, with cells rich in chromatophores. Still another variant is found in Mesogloea (Fig. 1, G) where the part beyond the meristem (*m*) is moniliform and composed of relatively short cells (*a*); it closely resembles one of the assimilators (para-physes) terminating the numerous laterals of the axial thread (*ax*) and con-stituting the major photosynthetic system of the plant. The fact that the meristem is surmounted by these diverse structures in genera (Mesogloea, Myriogloea, Acrothrix) which appear to belong to a single evolutionary series no doubt indicates their homology, and there does not seem to be any valid reason against applying the term trichothallic to all such infra-apical meri-stems, whether capped by a true hair or not.

Kylin (1940*a*), in a recent study, has shown that among the haplostichous Ectocarpales with cylindrical thalli (comprised in his Chordariales) there is considerable variety in the relative degree of importance of the trichothallic meristem in the axial threads. Thus, in Mesogloea (Fig. 1, G) the segments of the meristem (*m*) undergo considerable subsequent transverse division, whereas this is not so (Fig. 1, E) in the likewise uniaxial Sphaerotrichia (based on the *Chordaria divaricata* of Kuckuck, 1929, p. 72; cf. also Hygen, 1934) and in Acrothrix (Fig. 1, A); among multiaxial forms there is probably a similar restriction of meristematic activity in Chordaria (Kylin, 1940*a*, p. 61). In other words, in this haplostichous assemblage there is a manifest tendency to confine cell-division in the major axes to the region of the subapical meristem.

One family of haplostichous Ectocarpales, the Spermatochnaceae, exhibit apical growth which has also been recorded in a few species of Ectocarpus (Setchell and Gardner, 1922, p. 406; Oltmanns, 1922, p. 8). The uniaxial Nemacystus (Fig. 1, B) among Spermatochnaceae shows essentially the same

construction as Acrothrix (cf. Fig. 1, A and B), except that growth is effected by a hemispherical apical cell (*ac*), the segments of which undergo no transverse division. According to Kuckuck (1929, p. 65) the apical hair is shed in older plants of *A. gracilis* and, subsequent to this, the cells of the axial thread undergo enlargement only. If, however, the meristem remained functional, we should have reached what is tantamount to apical growth, and this apparently obtains in *A. novae-angliae* Taylor (1928), a species which comes very close to Nemacystus. There is therefore reason to believe that the apical growth of Spermatochnaceae is a secondarily acquired feature and has originated from the trichothallic growth seen in most of the haplostichous genera.

Many polystichous Ectocarpales seem to exhibit more or less diffuse growth, although it is known that, in certain genera (Stictyosiphon), the bulk of the meristematic activity in the older plant takes place below the apex (Kjellman, 1897, p. 206), while in others (Scytosiphon, Litosiphon) cell-division in later stages seems largely confined to a suprabasal position (Janczewski, 1875, p. 113; Kjellman, 1897, p. 199). There is commonly an apical hair (cf. Fig. 2, C and 3, D, E in Fritsch, 1942*a*). The Dictyosiphonaceae, however, have a dome-shaped apical cell, although some transverse division still occurs in the segments (Janczewski, 1875, p. 100; Murbeck, 1900, p. 4; Kuckuck, 1929, p. 87). There is evidence that, as in Spermatochnaceae, this apical growth is secondary and derived from a trichothallic mode of growth. Thus, young plants of *Dictyosiphon foeniculaceus* (Sauvageau, 1929, p. 259) bear an apical hair (Fig. 1, F, M, *h*) with the usual basal meristem (*m*), although this plays no part in the growth of the wider underlying portion which results from horizontal division of the apical cell. Sooner or later the hair is shed (Fig. 1, I) and, after this, the apical cell (*ac*) occupies the actual tip. It is therefore likely that evolutionary advance in Ectocarpales, and probably in Phaeophyceae as a whole, has been from intercalary to apical growth. The embryology of Fucus furnishes further evidence for this view (p. 81).

A comparable evolutionary progress is also recognizable among Ectocarpales in respect of reproductive features. This is exemplified by the evolution of heteromorphic alternation by divergent development of the two phases, and by the decreasing importance and final elimination of accessory reproduction of the sporophyte by the diploid swarmers from plurilocular sporangia. These tendencies have already been discussed in the second article (Fritsch, 1942*b*, pp. 543, 545), and it is only necessary to point out that both are clearly evident in those Ectocarpales (Spermatochnaceae, Dictyosiphonaceae) that have attained to apical growth. Attention may also be drawn to the fact that it is always the gametophyte that remains arrested at the primary filamentous stage and that no instance of isomorphic alternation is known among the polystichous forms.

In the majority of the haplostichous genera there is no marked soral aggregation of the reproductive organs of the sporophyte, although this is indicated in Ralfsia, Lithoderma, and various Spermatochnaceae. It is more evident among polystichous types (Punctaria, Petalonia, Scytosiphon, Asperococcus)

and appears in a specially characteristic manner in the series of genera grouped by Oltmanns (1922, p. 66) as Encoeliaceae. Here the sporangia tend to be aggregated around a central tuft of hyaline hairs (Fig. 2, A) which are commonly situated at the base of a depression (Fig. 2, B), a feature which is very marked in Soranthera, Chnoospora, and Colpomenia. Soral aggregation may be supposed to confer the advantage *inter alia* of mutual protection and, when such sori are associated with groups of hairs and paraphyses, of a means for localized nutritive supply. Other methods for the protection of the sporangia are realized in certain of the more specialized forms. Thus, in Chordaria the often enlarged and thickened end-cells of the short peripheral assimilators unite to form a protective cap, often supplemented by a layer of mucilage, over the developing sporangia; this foreshadows the condition typical of Laminariales. In Dictyosiphonaceae the isolated sporangia are superficial structures (Murbeck, 1900, p. 25) which become embedded in the surface by division of the surrounding cells.

Although most Ectocarpales are characterized by morphological isogamy, heterogamy is shown, for instance, by various species of Ectocarpus (*E. secundus* Kütz., Sauvageau, 1896, p. 388; *E. Mitchellae* Harv. (Fig. 2, L, M), Sauvageau, 1933, p. 67) and by Nemoderma (Kuckuck, 1912*a*, p. 122). A comparable trend is recognizable among Sphacelariales (cf. p. 77).

The following types of evolutionary advance can thus be recognized among Ectocarpales:

(i) Elaboration of a massive pseudoparenchymatous or parenchymatous thallus in diverse ways from the erect filaments of the primary heterotrichous stage. It may be recalled that in the more advanced orders the prostrate system is commonly suppressed (Fritsch, 1942*a*, p. 400), although retained in Tilopteridales and in certain Sphacelariales and Cutleriales.

(ii) A tendency for increasing restriction of cell-division to an intercalary, usually subapical, meristem and the final replacement of the latter by apical growth.

(iii) Differentiation of cortical and medullary regions in the parenchymatous (polystichous) types of thalli.

(iv) Differentiation of a superficial meristematic layer (meristoderm) bringing about increase in girth.

(v) Evolution of heteromorphic alternation by divergent development of the two phases.

(vi) Elimination of accessory reproduction by means of plurilocular sporangia on the sporophyte.

(vii) A tendency towards soral aggregation of the sporangia on the sporophyte, manifested more particularly in the more specialized types.

(viii) A tendency towards heterogamy.

It should be emphasized that all the evolutionary trends just noted find their culmination in other orders of Phaeophyceae. No better reasons could

be adduced for regarding the Ectocarpales as a relatively primitive series of Brown Algae exhibiting many experiments in evolution, some of which were more fully elaborated in the more advanced orders.

THE CLASSIFICATION AND INTERRELATIONS OF ECTOCARPALES

The Ectocarpales, as above defined, are primarily characterized by their heterotrichy and the lack of true oogamy, as well as by the comparatively simple organization of the thallus. There is of course a considerable range of structure, and no serious objection could be raised to the elevation of certain groups of families to ordinal rank, but the differences between them are only appreciable so far as vegetative structure is concerned and are scarcely of the nature usually accepted for distinguishing major systematic units. With the discovery of heteromorphic alternation in Dictyosiphon, a new taxonomic feature became available and the elevation of the Dictyosiphonaceae to ordinal rank was advocated (Taylor, 1922). Later Sauvageau (1926, p. 191) established the order Sporochnales, for which there is a sounder basis (see p. 74).

Recently Kylin (1933, p. 91; cf. also Taylor, 1936) has laid special stress on the nature of the life-cycle in the classification of Phaeophyceae as a whole, distinguishing the Isogeneratae with isomorphic from the Heterogeneratae with heteromorphic alternation. To the former he refers Ectocarpales (sens. limit.), Sphacelariales, Cutleriales, Tilopteridales, and Dictyotales; to the latter Laminariales, Sporochnales, Desmarestiales, Chordariales, Dictyosiphonales, and Punctariales; the last three are distinguished only on vegetative grounds. The Ectocarpales are restricted to those forms that show isomorphic alternation, viz. the Ectocarpaceae and some Myrionemataceae, and, since they include both simple filamentous types and crust-forming genera, they exhibit a considerable vegetative range. All the forms at present classed as Myrionemataceae are not isomorphic, and Kylin suggests that these may be reduced Mesogloeaceae. Even certain species of Ectocarpus show indications of heteromorphy (Fritsch, 1942b, p. 540).

The Ectocarpales with heteromorphic alternation are doubtless derived from simple filamentous types with an isomorphic life-cycle (Fritsch, 1942b, p. 545) by divergent development of the two generations. It is therefore questionable whether a classification of the simpler Brown Algae on the basis of their life-cycle is justified. Moreover, if such a classification were logically carried out, Cutleria would have to be grouped among Heterogeneratae and Zanardinia among Isogeneratae. It is of course clear that Cutleria is descended from an isomorphic ancestry (Fritsch, 1942a, p. 409), but that is equally true of Kylin's Chordariales, Dictyosiphonales, and Punctariales. It appears to the writer advantageous to include under one ordinal name all these heterotrichous types that display diverse experiments in the elaboration of a relatively unspecialized sporophyte, although a secure basis can be found for segregating the Sporochnaceae and Desmarestiaceae from the Ectocarpales of Oltmanns.

The different kinds of thallus-structure met with among Ectocarpales appear as variants of a common scheme, and the majority, if not all, seem to be directly derivable from simple heterotrichous filaments such as are represented among the Ectocarpaceae. This is shown by the great measure of uniformity displayed by the early ectocarpoid stages of thallus-development, in which the Ectocarpales differ from most of the other orders of Phaeophyceae. The diverse evolutionary series that seem to be distinguishable in fact appear as so many lines radiating out from a common stock (cf. the scheme on p. 84). Among the more important are the Myrionemataceae, Elachistaceae, Leathesiaceae (Corynophloeaceae), Mesogloeaceae (Chordariaceae), Spermatochnaceae among haplostichous forms; the Punctariaceae, Asperococcaceae, Encoeliaceae, and Dictyosiphonaceae among polystichous forms.

The crust-forming genera of Myrionemataceae (Ralfsia, Lithoderma) contrast markedly with the Elachistaceae in the mode of growth of the thallus and in the nature of the life-cycle, and the two no doubt represent distinct evolutionary series. Certain Myrionemataceae (Myrionema, &c.), with a looser construction, may, as already mentioned, be reduced members of other series, possibly of Mesogloeaceae. The latter and the Leathesiaceae have much in common, apart from the difference in the form of the mature thallus, and may well be closely related, while certain Spermatochnaceae (Nemacystus) may be directly derivable from the series represented by the Mesogloeaceae (cf. p. 66). It should be noted, however, that in Spermatochnus and its allies (Stilophora) we find a type of thallus-structure somewhat different from that exemplified by Nemacystus. The principal axes of Spermatochnus (Reinke, 1892, p. 53) become surrounded by a considerable pseudoparenchymatous envelope which originates from down-growing corticating threads produced from the basal and epibasal cells of the whorled laterals. The resulting structure departs appreciably from that typical of the Mesogloeaceae, from which Spermatochnus and Stilophora also differ in the way in which the sporangia are borne. It is not impossible that these genera may belong to a line of evolution distinct from that represented by the Mesogloeaceae and Nemacystus.

The diverse polystichous genera for the most part appear closely allied, and genera such as Phloeospora and Isthmoploea demonstrate the intimate relation to simple filamentous types. The Punctariaceae (Phloeospora, Litosiphon, Punctaria, &c.) and Asperococcaceae (Isthmoploea, Asperococcus, Petalonia, &c.) would seem to represent different evolutionary series originating from a source of this kind, while the Dictyosiphonaceae are possibly a side-branch from the line of evolution of the Punctariaceae, in which apical growth became established. The Encoeliaceae are almost certainly heterogeneous. It may well be that further investigation will show that certain polystichous forms are related to haplostichous types. Occasional longitudinal division occurs in diverse Ectocarpaceae (Pylaiella), and the tendency is evident in the multiseriate character of the plurilocular sporangia of many genera. Giraudya, which shows resemblances to Elachista with which it has usually been classed,

has threads with a parenchymatous structure and is essentially a polystichous type (Kylin, 1933, p. 93).

<center>TILOPTERIDALES</center>

Since the time when Reinke (1889) showed that Kjellman's *Scaphospora speciosa* (1877, p. 29; cf. also Brebner, 1897, p. 179) was altogether identical in vegetative features with *Haplospora globosa*, an isomorphic alternation between a sexual 'Scaphospora-phase' and an asexual 'Haplospora-phase' has usually been accepted. The supposed sexual plants bear large monosporangia, as well as plurilocular sporangia of a distinctive type (Fig. 2, D) liberating spermatozoid-like swarmers; these structures are generally interpreted as antheridia. The asexual plant produces only monosporangia which set free quadrinucleate monospores (Fig. 2, C) provided with a definite membrane. The monosporangia of the Scaphospora-phase, on the other hand, liberate naked uninucleate cells which are suspected of being ova. The sporangia on the two types of individuals also usually occupy a different position (Reinke, 1889, p. 127). Nienburg (1923) described synezesis and indistinct diakinesis stages during monospore-formation on the asexual plant (cf. also Dammann, 1930, p. 21), although the evidence for halving of the chromosome number is inadequate.

In *Tilopteris Mertensii* Reinke (1889, p. 156) observed 2, 4, or sometimes more nuclei in the clothed monospores liberated from plants bearing monosporangia only. He concluded that these plants belonged to the asexual phase of this species, while others previously recorded by Thuret (1855), with both monosporangia and plurilocular sporangia like those of Haplospora, were regarded as representing the sexual phase. Reinke thus assumed an isomorphic alternation for Tilopteris, comparable to that believed to occur in Haplospora, although in the former genus there is no difference either in position or form between the monosporangia of the asexual plant and the supposed oogonia of the sexual individual; no cytological data are available.

The hypothesis of Reinke just outlined has been accepted by several authorities (Kylin, 1917; 1933, p. 92; Oltmanns, 1922, p. 175; Kniep, 1928, p. 178). The evidence for the existence of an isomorphic alternation is considerable, but there are serious objections to the interpretation of the monosporangia on the sexual phase as oogonia. There is no indication that the supposed spermatozoids are attracted by the naked uninucleate monospores formed on the same individuals (Kuckuck, 1912b, p. 182), nor is there at present any evidence that these give rise to asexual plants. A further objection to the current hypothesis lies in the absence of any parallel for the complete similarity between monosporangia and female organs such as it postulates. Sauvageau (1899, p. 121) emphasizes the great range in size of the uninucleate monospores, a feature which is unusual in a sexual cell. Brebner (1897, p. 178), moreover, records the presence of antheridia and of uni- and quadrinucleate monospores on the same plant in Haplospora, although diverse subsequent workers have regarded this as an abnormality. The simple hetero-
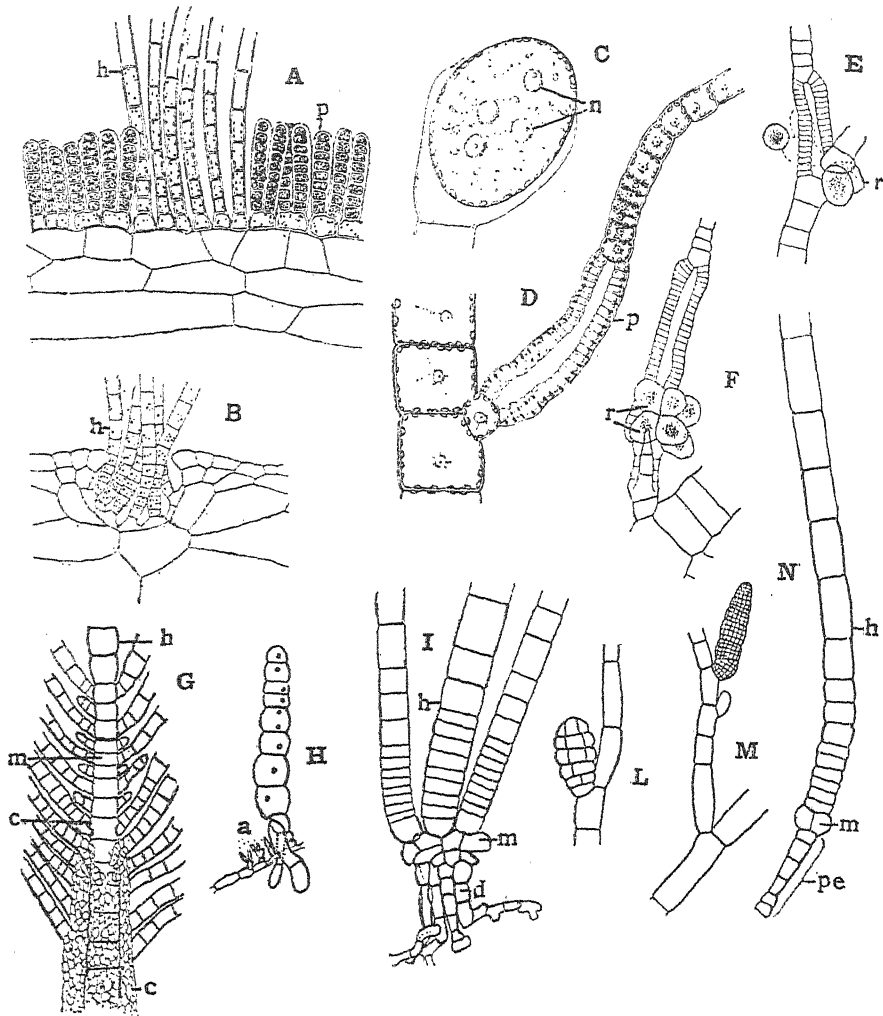
FIG. 2. A, *Chnoospora fastigiata* J. Ag., sorus of plurilocular sporangia. B, *C. obtusangula* (Harv.) Sond., section of thallus with hair-cavity. C, D, *Haplospora globosa* Kjellm.; C, liberation of quadrinucleate monospore from asexual phase; D, plurilocular sporangium (antheridium?). E, F, *Tilopteris Mertensii* (Smith) Kütz., plurilocular sporangia, with liberation of large reproductive cells (r) from lower compartments. G, *Desmarestia aculeata* (L.) Lamour., apex of growing plant. H, *Carpomitra costata* Batt., young proembryo arising from gametophyte. I, N, *Nereia filiformis* Zan.; N, proembryo with commencing differentiation; I, older stage. L, M, *Ectocarpus Mitchellae* Harv.; L, female, and M, male gametangia. a, antheridia; c, corticating threads; d, down-growing threads; h, hairs; m, meristem; n, nuclei; p, plurilocular sporangia; pe, pedicel of proembryo. (A after Boergesen; C, D, G after Reinke; E, F after Dammann; H, L, M after Sauvageau; the rest after Kuckuck.)

trichous filamentous habit of Tilopteridales also militates against the likelihood of their having attained to oogamy, since all oogamous Phaeophyceae exhibit specialization in vegetative structure.

A somewhat different complexion has been put on the problem by the demonstration (Dammann, 1930, p. 15; cf. also Bornet, 1891, p. 368) that the plurilocular sporangia of Tilopteris may liberate both spermatozoids and, from the lower compartments (Fig. 2, E, F), larger amoeboid dark-brown units (r) which are possibly female gametes. The latter resemble the swarmers (Sauvageau, 1897, p. 33) set free from the plurilocular sporangia of Acinetospora (Bornet, 1891, p. 356; Schussnig, 1928, p. 166) which differ from the supposed antheridia of the other two genera in possessing relatively few and large compartments. The monospores of Acinetospora are always uninucleate and provided with a membrane before liberation (Kuckuck, 1895b, p. 295; Sauvageau, 1899, p. 110). Organs resembling antheridia have not so far been found in this genus, but in view of the rarity of Tilopteridales and of the progressive discovery of different kinds of reproductive structures among them, it is not impossible that they may yet be found. It seems not improbable that the members of this order may be anisogamous forms, producing the two kinds of gametes in the same (Tilopteris) or distinct (Acinetospora) gametangia. If this proved to be so, the monosporangia on the sexual individuals would be relegated to the position of accessory asexual reproductive organs, an interpretation which Sauvageau (1899, 1928) has always maintained and which is also supported by Schmidt's (1940) observations on Acinetospora.

In vegetative structure the Tilopteridales are but little removed from the simple Ectocarpaceae; like them they exhibit heterotrichy and trichothallic growth, and the only advance lies in the tendency towards longitudinal septation of the older threads. The latter feature is not seen in Acinetospora, which is also exceptional in having unilocular sporangia producing swarmers of a normal type. Kylin (1917, p. 306) would include this genus in Ectocarpaceae, although it appears to show direct points of affinity with other Tilopteridales, especially in the possession of monosporangia. It is the presence of these organs that chiefly distinguishes the members of this order from Ectocarpaceae. The monosporangia are best interpreted as modified unilocular sporangia (Schussnig, 1928, p. 169) which appear to have altogether replaced the usual type in Haplospora and Tilopteris. Other indications of specialization are seen in the peculiar structure of the supposed antheridia of these two genera.

Masonophycus (Setchell and Gardner, 1930, p. 141), which is tentatively referred to this order, is distinguished by producing sporangia containing four cruciately arranged spores. It is at present so incompletely known that its reference to a distinct family (Schmidt, 1937) seems premature. Its discovery strengthens a suspicion that there may be a remote affinity between Tilopteridales and Dictyotales in which likewise asexual propagation by zoospores is replaced by that by motionless spores.

CUTLERIALES

The mode of origin of the thallus in Cutleria and Zanardinia shows considerable analogies with that seen in Elachistaceae. In view, however, of the probable simple filamentous nature of the gametophyte in the latter, the resemblance in vegetative features is hardly likely to imply any close degree of affinity. As far as present evidence goes, the Cutleriales must be regarded as a direct development from the heterotrichous ectocarpoid stock with its trichothallic growth, isomorphic alternation, and tendency towards heterogamy. Cutleria is remarkable in showing in its two phases both the haplostichous and polystichous types of construction, and in the Aglaozonia-stage furnishes the only example of a true parenchymatous structure in an encrusting type.

SPOROCHNALES AND DESMARESTIALES

The group of highly derived uniaxial genera comprised in the Sporochnales is distinguished not only by its oogamy, but also by the unique mode of thallus-formation which has become clear through the researches of Sauvageau (1926, 1927*a*) and Kuckuck (1929). The zygote gives rise to an erect-growing proembryonal thread (Fig. 2, H), exhibiting diffuse growth and attached by basal rhizoids. Soon this proembryo becomes differentiated (Fig. 2, N) into (*a*) a lower part or pedicel (*pe*) which may reach some length in Carpomitra (Fig. 3, B) and (*b*) an upper part which develops a basal meristem and constitutes the first assimilatory hair (*h*) of the future tuft. The single flat cell (Fig. 2, N, *m*), situated between these two regions, sooner or later undergoes longitudinal division in various planes to form a progressively broadening horizontal meristem (Fig. 2, I, *m*), from the individual cells of which the mature thallus originates as successive downwardly growing threads (*d*) which cover the lower part (pedicel) of the proembryo (Fig. 3, C), and in part spread out on reaching the substratum. Successive protuberances arise on the upper side of the meristem and give rise to the characteristic tuft of assimilatory threads (Fig. 3, D, *h*) that crowns the summit of each growing branch. Although primarily uniaxial, the mature thallus can be regarded as a multiaxial one (cf. Fig. 3, D), but originating in a radically different way from that met with in the haplostichous Ectocarpales.

The mode of formation of the mature thallus shows certain analogies with the process of cortication in Desmarestiales, where the primary axes become enveloped in downwardly growing cortical threads, and the analogy is heightened in certain instances observed by Sauvageau (1926, p. 167) in Carpomitra, in which more than one transverse meristem arises in the course of the proembryonal thread. Berthold (Oltmanns, 1922, p. 48) believed to find in the mature structure of the sporophyte of Sporochnales indications of a relationship with Mesogloeaceae, whilst Oltmanns himself refers especially to Myriogloea, but it is difficult to find any grounds for assuming such an
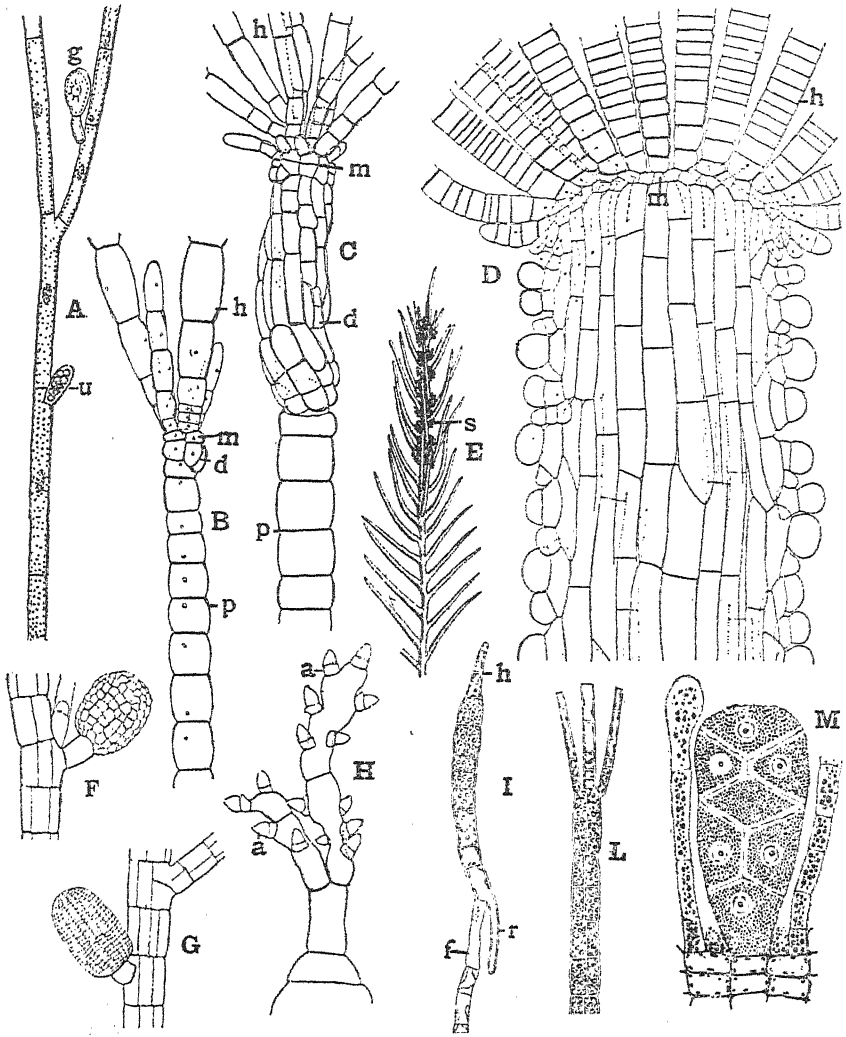
FIG. 3. A, *Choristocarpus tenellus* (Kütz.) Zanard. B, C, *Carpomitra costata* Batt.; B, young, and C, older proembryos. D, *Nereia filiformis* Zan., longitudinal section of apex of growing plant. E, *Halopteris scoparia* (Kütz.) Sauv., fertile spikelet. F, G, *Sphacelaria hystrix* Suhr, the two types of gametangia. H, *Nereia filiformis* Zan., part of a male prothallus. I, L, *Chorda filum* (L.) Stackh.; I, very young plant attached to prothallus (*f*); L, apical part of a somewhat older stage. M, *Zonaria Farlowii* Setch. and Gardn., sporangium with paraphyses. *a*, antheridia; *d*, down-growing threads; *g*, propagule; *h*, hair; *m*, meristem; *p*, pedicel of proembryo; *r*, rhizoid; *s*, sporangia; *u*, unilocular sporangium. (A, D, after Kuckuck; E after Reinke; I, L after Kylin; M after Haupt; the rest after Sauvageau.)

affinity. The Sporochnales are a highly specialized heteromorphic group with a structure for which it is difficult to find any exact parallel.

The Desmarestiales are uniaxial forms which exhibit trichothallic growth and acquire. a massive construction by a process of cortication (Fig. 2, G) which shows analogies to that found in Spermatochnus (Jönsson, 1901, p. 7). More or less long-continued meristematic activity on the part of the surface-cells of the resulting envelope leads to the formation of a cortex of considerable width around the primary axial cells; in Desmarestia there are further complications. Arthrocladia shows whorled, the more specialized Desmarestia distichous branching, often combined with marked flattening of the mature thallus. Despite some analogies in structure, no direct affinity with Spermatochnus or its allies is likely.

The gametophytes of these two orders in general characteristics resemble those of the heteromorphic Ectocarpales, although they bear oogonia and antheridia which, especially in Desmarestia, closely resemble those of Laminariales. This led Schreiber (1932, p. 579) actually to include the Desmarestiaceae in the latter order, which is quite inadmissible in view of the complete dissimilarity in the structure of the sporophytes. Interest attaches to the fact that in Nereia (Fig. 3, H) the antheridia (*a*) are two-celled (Sauvageau, 1927*a*, p. 360) which possibly indicates a step in reduction from the plurilocular type. The gametangia in many heteromorphic Ectocarpales are few-celled. As already pointed out (Fritsch, 1942*b*, p. 546), the ectocarpoid character of the gametophytes in these two orders justifies the assumption of an ultimate isomorphic origin for both orders and this must no doubt be sought in simple filamentous types. As compared with the many series of Ectocarpales emanating from such a source, Sporochnales and Desmarestiales stand out by the greater degree of specialization in thallus-structure and by their oogamy.

SPHACELARIALES AND DICTYOTALES

In their pronounced apical growth, characteristic segmentation, and probable isomorphic alternation, the Sphacelariales form a very clearly defined group which is almost certainly homogeneous. Several of the less specialized Sphacelarias (*S. pulvinata, S. bracteata*), in which relatively little longitudinal division occurs, and the possibly reduced Sphacella (Reinke, 1891), in which such division is scanty and may be altogether lacking, depart but little from a simple Ectocarpus-like type and warrant the assumption of an ancestry among heterotrichous filamentous forms that early acquired apical growth. A simple filamentous type with apical growth, that is usually brought into relation with this order and is indeed definitely referred to it by Kylin (1917, p. 306), is Choristocarpus (Fig. 3, A; Falkenberg, 1879, p. 65; Kuckuck, 1895*b*; 1929, p. 13; Sauvageau, 1931, p. 158), but it is not known whether this is heterotrichous or not. Choristocarpus is, however, probably isomorphic, since individuals bearing uni- (Fig. 3, A, *u*) and plurilocular sporangia respectively are known. The former also produce oblong, 2- (sometimes 1- or

3-) celled propagules (Hauck, 1887; Kuckuck, 1895*b*, p. 310) borne on a unicellular stalk (Fig. 3, A, *g*) from which the whole of the mature structure is detached. Although less specialized in form than those of the Sphacelarias, their presence increases the probability that Choristocarpus is to be regarded as a simple member of this series (cf. also Kuckuck, 1895*b*, p. 316). It may well represent an offshoot from the main line of evolution.

The cell-contents of the propagules of Choristocarpus resemble those of the monospores of Tilopteridales, and this led Kuckuck (1895*b*, p. 311) to homologize the two structures. The monospores of the latter are, however, clearly of the nature of spores and the organs which produce them sporangia. While Tilopteridales and Sphacelariales resemble one another in the retention of heterotrichy and in their probable isomorphic life-cycles, it may be doubted whether there is any close affinity between them.

The numerous modifications in vegetative structure that occur among Sphacelariales are all traceable to a common plan. A significant development was that leading to the holoblastic branching of Halopteris, Phloeocaulon, and Ptilopogon (Sauvageau, 1903, p. 334), which results in the production of axillary initials from which in the fertile regions the sporangia usually arise. By division of the initial smaller or larger groups of axillary sporangia are formed. In *Halopteris scoparia* (Sauvageau, 1904, p. 365), *H. hordacea* (Sauvageau, 1904, p. 426), and Phloeocaulon (Reinke, 1891, p. 30) such fertile systems are aggregated in definite spikelets (Fig. 3, E) in which the subtending laterals appear as bracts, whilst in the last there is produced on either side of each axillary sorus a short branch which occupies the position of a bracteole.

While diverse Sphacelarias, as well as *Cladostephus spongiosus*, are certainly isogamous, there is considerable presumptive evidence of heterogamy in some species of Sphacelaria (Fig. 3, F, G) and in other more advanced forms, viz. *Halopteris filicina* (Sauvageau, 1903, p. 419), Phloeocaulon, and Ptilopogon (Sauvageau, 1904, pp. 462, 467, 479), in all of which two types of plurilocular sporangia have been recorded. Whether the members of this order have actually attained to oogamy, as Sauvageau (1904, pp. 370, 428) suspects may be the case in *Halopteris scoparia* and *H. hordacea*, must await further investigation. It is to botanists in the Southern Hemisphere, where this order appears to attain its maximum development, that we must especially look for an elucidation of these matters.

The less specialized members of Sphacelariales stand nearer to the ancestral ectocarpoid stock than do the members of any other order of Phaeophyceae. This is shown by their heterotrichy, by their relatively simple structure, and by the presence on the diploid phase of plurilocular sporangia subserving accessory reproduction.

The likewise isomorphic Dictyotales exhibit advance rather in reproductive than in vegetative organization and seem to represent a specialized line that has departed rather widely from the normal type. The reproduction of the asexual phase by tetraspores, combined with the fact that the male cells were long thought to be motionless, led to the assumption of an affinity with Red

Algae (Falkenberg, 1882, p. 169), an erroneous view which is still occasionally propounded. The pigmentation of the chromatophores, what is known of the metabolic products (fucosan vesicles), the plurilocular nature of the antheridia, and the general structure of the spermatozoids as established by Lloyd Williams (1904, p. 190), all betray features characteristic of Phaeophyceae and speak emphatically against any affinity with Rhodophyceae. Even the tetra-sporangia are no doubt to be regarded merely as specialized unilocular spor-angia (Kylin, 1933, p. 76), and in this connexion the frequent production of 8 spores in Zonaria (Fig. 3, M; Sauvageau, 1905, p. 68; Haupt, 1932, p. 248) is significant. Diverse Ectocarpales (*Ectocarpus granulosus, Pylaiella fulvescens,* Kuckuck, 1912b, p. 175) form only few spores in the unilocular sporangia, and in such instances they often show little mobility, apart from amoeboid move-ments.

It is, however, difficult to trace any direct affinities between Dictyotales and other Phaeophyceae. With the Sphacelariales, apart from the apical growth and the isomorphic alternation, they appear to have little in common, and it is difficult to find any satisfactory evidence for a derivation of Dictyotales from this order, as advocated by Smith (1938, p. 229). Sauvageau (1905, p. 80) believed in some affinity with Cutleriales owing to the close resemblance in habit and structure between certain species of Zonaria (*Z. variegata*) and the Aglaozonia-stages of Cutleria (especially the unassigned *A. tanariensis,* Howe, 1920, p. 591; Boergesen, 1926, p. 77); it seems probable, however, that such resemblances are purely homoplastic. On present evidence the Dictyotales must be supposed to have originated directly from the primitive filamentous stock, along a line in which apical growth and reproductive specialization was early established.

LAMINARIALES AND FUCALES

These two orders are of special interest, not only because they exemplify the potentialities for somatic advance inherent in the parenchymatous thallus-organization, but because both in stature and in numbers of individuals they represent the dominant vegetation of the colder seas. There can be little question that the Laminariales represent an extreme development of the type of construction met with in the polystichous Ectocarpales. The gameto-phytes, except in their oogamy, do not differ fundamentally from those of the heteromorphic Ectocarpales and find a complete parallel in those of the haplostichous Desmarestiales. The sporophytes can be regarded as showing to an extreme the elaboration that is hinted at in the polystichous Ectocar-pales. Except in the absence of a prostrate system, young stages of Chorda (Fig. 3, I, L) closely resemble those of many of the latter, the similarity extend-ing even to the presence of the customary apical hair (Kylin, 1933, p. 72). The greater degree of anatomical specialization only becomes evident in later stages, with the differentiation of 'trumpet-hyphae' around the medullary cavity. The pronounced superficial meristematic activity and the specialized sori are both already foreshadowed among some of the parenchymatous Ectocar-

pales. The relatively simple anatomical structure, as contrasted with that of other Laminariales, may well be due to the absence of an expanded lamina which, when present, probably necessitates a more elaborate mechanical construction, such as is supplied by the medulla of the stipe of most Laminariales. A closer connexion with any of the existing series of polystichous Ectocarpales is unlikely, and the Laminariales probably represent a separate parenchymatous stock originating from primitive filamentous forms.

There are many similarities in anatomical structure between Laminariales and Fucales, though the latter do not attain to as high a differentiation as is realized in certain members of the former order. Moreover, the possibility of any direct affinity is ruled out by the altogether different method of growth. It is in the reproductive organization and in the peculiar life-cycle of Fucales, however, that the most significant contrasts between the two orders are to be found. In the Fucales the sex organs are borne on the diploid generation and reduction is effected during gametogenesis (cf. the diploid Siphonales among Chlorophyceae).

Various features (dichotomous branching, production of 8 ova in the oogonium, the embryology) render it probable that Fucus exemplifies a relatively primitive type within the order, although in other respects this genus exhibits a degree of specialization that shows that it has departed widely from the ancestral type. Nienburg's (1931; cf. also Farmer and Williams, 1898, p. 640) study of the embryology of Fucus brought to light diverse features that indicate the relatively primitive status of the genus and at the same time shed some light on the probable origin of Fucales, as well as on some of their peculiar characteristics. The embryo of Fucus differs from that of most of the other Fucales that have been studied in the formation of a number of transverse septa before longitudinal division sets in (Fig. 4, A), as well as in the fact that the basal part of the germling is developed as a multi-septate unbranched rhizoid[1] (Fig. 4, B, *r*), later supplemented by others. More or less regular cell-division proceeds for a considerable period in the body of the embryo (Fig. 4, B), but sooner or later, while the latter is still globular, one of the top cells becomes distinguished by a specially conspicuous nucleus. This cell grows out into a hair (Fig. 4, C, *h*) which develops a meristem (*m*) near its base above the initial cell which persists unaltered. At this stage there is some similarity with young plants of Chorda or Scytosiphon, for example. During the differentiation of the hair the surrounding cells separate from it (Fig. 4, C) and initiate a funnel-shaped apical depression which rapidly widens. By degrees other adjoining cells, occupying the sides of this depression, successively grow out into hairs (Fig. 4, D, *h*) and, as this happens, the first-formed hair gradually disorganizes, except for its basal cell (*i*) which becomes converted into the 3-sided apical cell of the embryo (Fig. 4, G, *a*). By means of this the further growth is largely effected, although a superficial meristoderm (Fig. 4, E, G, *s*) is early established and contributes to increase in girth behind the apex.

[1] A multicellular rhizoid has also been recorded in certain Japanese Fucales (Inoh, 1935).
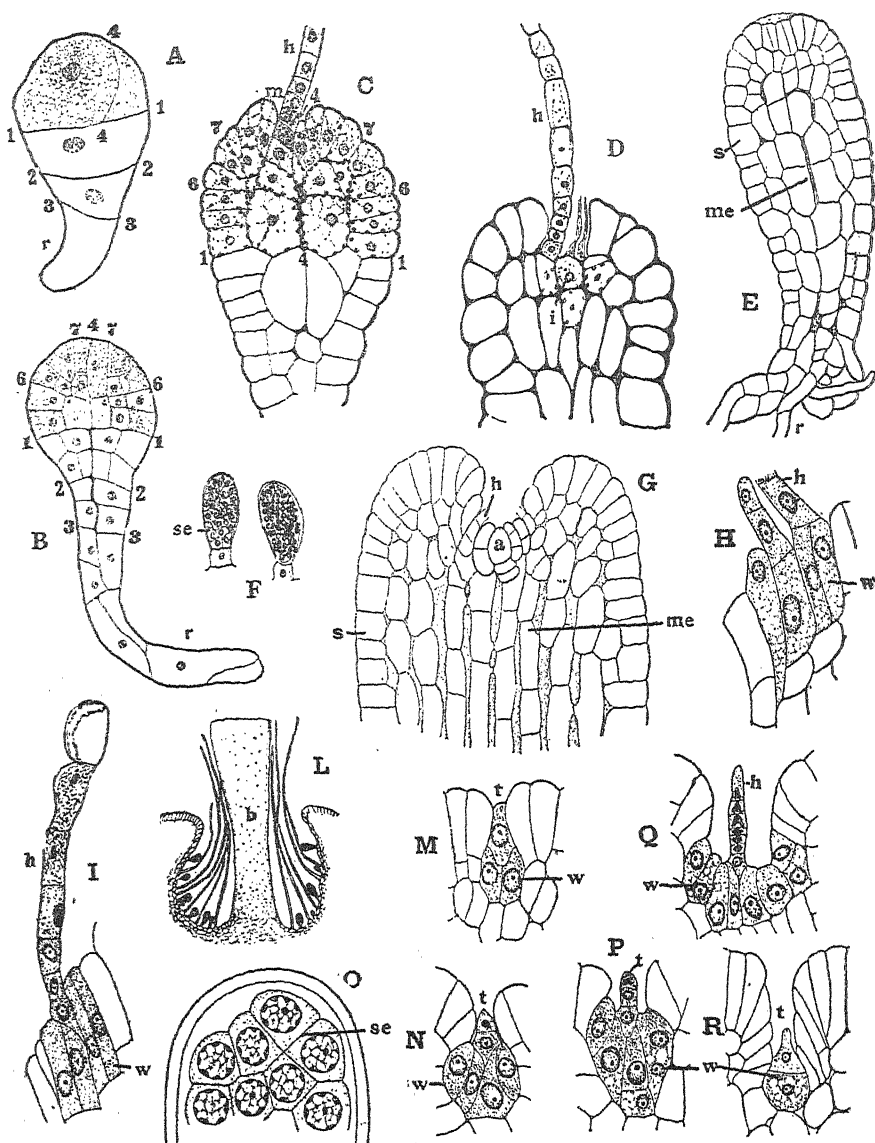
FIG. 4. A–E, G, *Fucus vesiculosus* L., various stages in development of embryo; A, the first septa (1–4); B, older germling showing the septate rhizoid; C, formation of first hair and differentiation of apical pit; D, differentiation of apical cell from initial (*i*) of first hair; E, longitudinal section of somewhat older plant, commencing differentiation of medulla (*me*); G, ditto, later stage. F, *Pelvetia fastigiata* (J. Ag.) De Toni, single antheridia on wall of conceptacle. H, I, *Himanthalia lorea* Lyngb.; H, basal cell of right-hand hair (*h*) within apical pit of receptacle commencing to divide to form the conceptacular lining (*w*); I, later stage of same. L, *Notheia anomala* Bail. et Harv., outgrowth of branch (*b*) from base of female conceptacle. M, N, P, Q, *Cystoseira barbata* Ag., successive stages in development of conceptacle. O, R, *Fucus serratus* L.; O, part of almost mature antheridium; R, early development of conceptacle. *a*, apical cell; *h*, hair; *m*, meristem of hair; *me*, medulla; *r*, rhizoid; *s*, surface-layer (meristoderm); *se*, septa in antheridia; *t*, tongue-cell; *w*, wall of conceptacle. (E, G after Oltmanns; F after Moore; L after Gruber; O after Kylin; the rest after Nienburg.)

The method of development of the apical cell suggests a derivation from a trichothallic meristem and shows appreciable analogy to the course of events already noted in Dictyosiphon (p. 67). It implies that, as in some Ectocarpales, the apical cell has probably been secondarily acquired. In the immediate allies of Fucus—Pelvetia (Oltmanns, 1888, p. 24), Ascophyllum—the apical pit does not produce any hairs, so that in these, evidently derived, types the symptoms of the mode of origin of the apical cell have been lost. In the later development of the embryo of Fucus the place of the 3-sided is taken by the characteristic 4-sided apical cell (Woodworth, 1888), which is peculiar to Fucaceae and is probably to be related to the usual flattening of the thallus.

The mode of origin of the primary apical cell in Fucus presents considerable analogies with the early development of the conceptacles which are such a characteristic feature of Fucales. The layer of cells lining the inner surface of the conceptacle is to a larger or smaller extent derived from the products of division of an initial cell which appears in all instances to be homologous with the basal cell of a hair, although the latter is suppressed to a varying extent. It is well developed in Himanthalia (Fig. 4, H, 1; Bower, 1880, p. 45; Oltmanns, 1888, p. 80; Nienburg, 1913, p. 12), but reduced to the peculiar 'tongue-cell' (Fig. 4, M, N, P, Q, *t*) in Cystoseiraceae (Valiante, 1883, p. 11; Nienburg, 1913, p. 3; Doubt, 1928), Sargassaceae (Simons, 1906; Delf, 1939*b*, p. 133; Dawson, 1940, p. 289), and Fucus (Fig. 4, R; Nienburg, 1913, p. 7). The common presence in young plants of many Brown Algae of an apical hair, at the base of which lies the meristem or at the base of which it subsequently develops (cf. Nienburg, 1931, p. 60), shows that in this class the hair is a common precursor or associate of meristematic activity. The conceptacle can thus be interpreted as a specially modified branch or branch-system. In other words, the conceptacles may be regarded as special developments of the pits that harbour the apical cells in Fucales, these pits here undergoing appreciable enlargement and the products of division of the initial being diverted to function in the formation of the sex organs. This view receives some support from the fact that in the peculiar genus Notheia (Fig. 4, L) the lateral branches (*b*) actually arise from the inner lining of the conceptacle (Gruber, 1896, p. 7; Barton, 1899, p. 419). A similar origin has been noted in salt-marsh forms of *Fucus ceranoides* (Skrine, Newton and Chater, 1932, p. 775), although in neither instance is it known whether the lateral actually originates from the basal cell of a hair.

Various attempts have been made to relate the conceptacles of Fucales to the more or less clearly marked cavities, associated with groups of hairs and reproductive organs (Fig. 2, A, B), commonly met with in the Encoeliaceae (cf. Oltmanns, 1922, p. 216). It is to be noted, however, that as a general rule the hairs alone originate within these cavities, while the associated reproductive organs stand around them on the free surface of the thallus (Fig. 2, A). Moreover, there is no evidence that the 'conceptacles' of Encoeliaceae arise in the way characteristic of Fucales, and the account of their development given by Sauvageau (1927*b*, p. 329) in Colpomenia indicates a totally different mode of

origin. These considerations render any direct connexion with Encoeliaceae improbable.

Many authorities have assumed some measure of relationship between Laminariales and Fucales which have been supposed to represent lines diverging from a common ancestral source comprising forms like the Ectocarpales. In their anatomical structure the two orders certainly exhibit a culmination of tendencies which are already indicated among the polystichous Ectocarpales. The frequent reduction, in cultures, of the female gametophyte of Laminariales to a unicellular condition, a state of affairs which is apparently the rule in Saccorhiza, has been regarded as symptomatic of a tendency towards retrogression of the haploid phase which has culminated in the condition found in Fucales (Kylin, 1918, p. 57;[1] Svedelius, 1921, p. 184; 1927, p. 372; 1931, p. 44; Kniep, 1928, p. 185). The first two nuclear divisions in the oogonium are interpreted as a tetrad-division within a sporangium (cf. also Strasburger, 1906, p. 4) in which, however, the spores are not individualized. Each, after a brief resting period, gives rise to two potential ova which represent all there is of the female gametophyte. It is not so easy to extend this interpretation to the antheridium.

It may be doubted, however, whether any such detailed attempts at homology are profitable, since there can be no question of any direct derivation of Fucales from Laminariales or other specialized order of Phaeophyceae. It seems more probable that the reproductive mechanism of Fucales has originated from the occasional tendency for the, normally asexual, zoospores of the diploid generation to behave as gametes, evident in diverse evolutionary lines among the less specialized Brown Algae (see Knight, 1931, p. 32; Fritsch, 1942b, p. 540). Such a view does not involve the assumption of a reduced gametophyte, although, like the hypothesis outlined above, it ultimately derives the sex organs of Fucales from unilocular sporangia. A sporangial origin for the oogonium is suggested by the fact that the Fucales are the only Brown Algae which produce more than a single ovum in the oogonium—indeed, apart from the highly aberrant genus Sphaeroplea, the Fucales are in this respect unique among holophytic oogamous plants.

In recent years Kylin (1933, p. 77; 1937, p. 28; 1938, p. 437), laying stress on the differences in the relative lengths of the flagella in the spermatozoids of Fucales on the one and in the swarmers of Ectocarpales on the other hand, as well as on the possible plurilocular character of the antheridia (cf. Fig. 4, o) and oogonia in the Fucaceae, disputes the probability of a direct derivation of Fucales from forms resembling present-day Ectocarpales. The evidence on the former point is conflicting (Delf, 1939a, p. 227), nor does it appear to be very significant. The septa apparent within the ripe antheridia and oogonia are not a result of progressive division as in a plurilocular sporangium, but appear only at maturity and seem to have much in common with the mucilaginous septa recorded between the ultimate units in the unilocular sporangia of diverse Ectocarpales (Kuckuck, 1912b, p. 164). The occurrence of the

[1] This hypothesis was later abandoned by Kylin.

antheridia in Fucales on richly branched hair-like growths is clearly a secondary feature, since the branch-system originates sympodially from the stalk-cell of the primary antheridium (Bower, 1880, p. 49; Yamanouchi, 1909, p. 176), which, like the oogonium, is borne directly on the wall of the conceptacle. The occurrence of single antheridia (Fig. 4, F) has been frequently recorded (Holtz, 1903, p. 40; Moore, 1928, p. 430; Barton, 1899, p. 421; 1891, p. 225).

In the writer's opinion the Fucales must have taken their origin in the evolutionary series exemplified by the present-day parenchymatous (polystichous) Ectocarpales, among which replacement of trichothallic by apical growth occurs. The behaviour of zoospores from unilocular sporangia as gametes, which is apparently only an occasional phenomenon in Ectocarpales, must have become fixed in this line of descent and been followed by a progressive development of heterogamy (cf. also Kylin, 1938, p. 439; 1940*b*, p. 308). There can be no doubt, however, that the Fucales have a long history behind them, and all intermediate forms seem to have become extinct. It may be questioned whether there is any direct connexion with Laminariales, the undoubted resemblances being homoplastic and the outcome of tendencies inherent in a plant-body possessing a parenchymatous organization. Delf (1939*a*, p. 236), in assuming an affinity with Mesogloeaceae, overlooks the fundamental differences in basic structure. The filamentous character of the medulla in Fucales is derived from a primary parenchymatous construction by gelatinization of the walls of adjacent files of cells (Fig. 4, E, G). There is no evidence whatsoever that the parenchymatous type of thallus in Phaeophyceae is derived from the pseudoparenchymatous one, although Schussnig (1938, p. 265) seems to have some such hypothesis in mind. The development of the mature thallus, both in Fucales and Laminariales, advances centripetally, whereas in Mesogloeaceae and other haplostichous types it takes place centrifugally.

The majority of the less specialized Fucales are characteristic of the colder seas of the Northern and Southern Hemispheres, whence they have invaded subtropical and tropical areas. That such widespread genera as Cystoseira, Cystophyllum, and Sargassum are comparatively recent and still in course of vigorous evolution is shown not only by their morphological features and the presence of a single ovum in the oogonium, but also by the multiplicity of ill-defined species.

General Conclusions and Summary

The previous considerations tend to indicate that the diverse specialized orders of Phaeophyceae have no direct affinities with one another and that they must be regarded as so many separate evolutionary lines emanating from simple filamentous forms with an isomorphic life-cycle (see the scheme on p. 84). In some (Cutleriales, Sphacelariales, Dictyotales) both generations have undergone specialization and the isomorphic life-cycle has been retained, but in Sporochnales, Desmarestiales, and Laminariales, as in so many Ectocarpales, only the diploid generation has become elaborated. It is noteworthy

that it is in these heteromorphic forms that the greatest degree of somatic differentiation has been attained. The Fucales are to be regarded as comparatively isolated forms, which have lost a definite phase-alternation as a
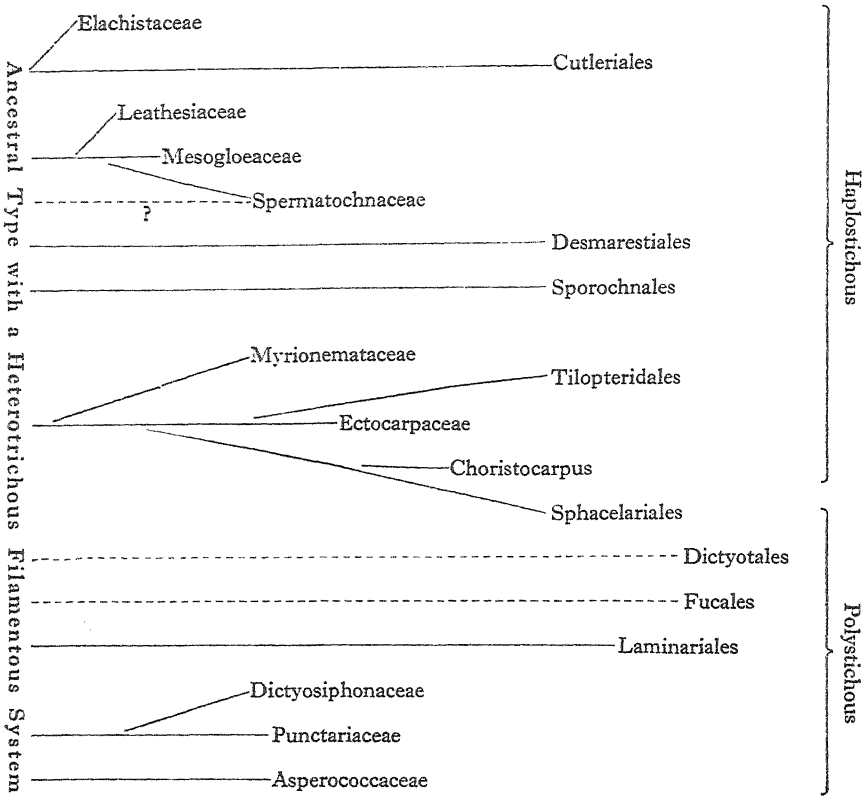
Scheme of Evolution of Phaeophyceae.

result of permanent adoption of the tendency of the zoospores of the diploid phase to behave as gametes which is evident in diverse of the less specialized orders of Phaeophyceae.

Many of the features that serve to distinguish the various orders of Brown Algae are recognizable in varying degrees in the sporophytes of the heteromorphic Ectocarpales, but such resemblances can only rarely, if ever, justify a belief in any direct connexion. The diverse series of Ectocarpales represent the outcome of various experiments in the production of a diploid thallus by elaboration of the heterotrichous system, and some may perhaps be relatively recent developments, but practically none of them has resulted in the degree of somatic advance that distinguishes the oogamous orders. All of these are clearly characterized, and the detailed course of evolution that most of them (Sporochnales, Desmarestiales, Laminariales, Fucales, Dictyotales) have fol-

lowed is obscure because of the absence of all intermediate forms, which is a result of the imperfection of the fossil record. Apart from Dictyotales and Fucales, however, it is not difficult to visualize a direct filamentous origin, although the Cutleriales, Tilopteridales, and Sphacelariales alone admit of a closer linkage with the ancestral type, and these are orders that have tended to retain an isomorphic life-cycle.

The sharp segregation of the heteromorphic orders (especially Laminariales and Fucales), as well as of the isomorphic Dictyotales, is indicative of a long course of evolution. The relatively close degree of interrelationship that is recognizable among many Florideae is lacking in Phaeophyceae, and the facts imply that the Phaeophyceae as a whole are a comparatively ancient group that is possibly still in course of vigorous evolution at the present day. Among the many lessons which they teach us, the most important are perhaps the derivation of a heteromorphic from an isomorphic life-cycle and the great possibilities for somatic advance inherent in the parenchymatous type of plant-body. It should be emphasized, however, that the detailed course of evolution of the latter among Phaeophyceae has followed paths which differ in many important respects from those that led to the evolution of archegoniate plants.

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The Effect of Ringing and Transpiration on Mineral Uptake

BY

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THE bromide ion, applied as inorganic bromides, has now been used as a convenient indicator ion in many investigations on salt uptake by cells. To extend the use of bromides to the elucidation of the transfer of salts from root to shoot was an obvious development. Since 1930 experiments of this kind have in fact been made both in England and in the U.S.A. and a large body of evidence is now available.[1] Publication of the results was deferred to permit the full investigation of the many facets which this complex problem presents, for it involves, as well as movement, the accumulation of bromide in the tissues with all that this entails. In fact the point of view which emerges from the work is that movement is often limited by the factors which control salt accumulation. Some references to the work have already been made (Curtis, 1935; Clements, 1938; Steward, 1935; Stout and Hoagland, 1939). Pending the full presentation of the results the following comments may be made on the paper of Phillis and Mason (1940), who have used bromide in their work on cotton plants.

Phillis and Mason concluded that 'the removal of a ring of bark depressed uptake of bromide within a period of a *little over two hours from ringing*' (not authors' italics). The data (Tables 1 and 2) upon which this conclusion was based are the results of one experiment. The design of this experiment was unsymmetrical, and it seems that the implications of this asymmetry have not been sufficiently appreciated. On re-examination it will be clear that the conclusion does not follow from the data which Phillis and Mason present in their paper.

Phillis and Mason grew cotton plants in sand, two plants in each container. As grown they were stated to be closely alike, although no measure of the difference between the plants in a container or of the variability between containers was given. There were visible differences between the plants, for they were identified as the taller or the shorter of the pair, and as such were allotted to the treatments which were described. From their procedure it is apparent that Phillis and Mason hoped they would eliminate the differences as between the paired plants grown in a container and also between the different con-

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[1] A. G. Steward investigated the uptake and movement of bromide in *Cucurbita Pepo* in the Botany Dept., University of Leeds, in 1930, and the experiments were later continued in the Division of Plant Nutrition, University of California. Hoagland and his assistants have also investigated the factors which control movement of bromide in a variety of angiosperms. At Birkbeck College, University of London, J. A. Harrison investigated the absorption and movement of bromide in Populus, and in this work on trees material and assistance were obtained from the Botany Dept. of the University of Leeds.

tainers. The plants assigned for treatment were in batches so composed that, if the differences between paired plants were constant for all containers and if the design of the experiment had been a symmetrical one, they would have been balanced *with respect to the visible property used in their selection*. That this end was not achieved is shown by the procedure, which was briefly as follows.

After eight weeks of growth twenty-eight selected containers were divided into four groups. The unit for sampling and treatment was a batch of seven plants. Two batches were drawn from each group by assigning the taller plant from the first container to one batch and the shorter to the other. At the next container the procedure was reversed and so on. In this way a group of containers supplied two batches of initial plants or else one batch of ringed and one of normal plants.

Clearly the two contrasted batches of plants selected from each group *were not identical*, for in one case they included four relatively tall plants (4T) plus three relatively short ones (3S), and in the other the arrangement of 'talls' and 'shorts' was not only reversed (3T+4S) but the contrasted tall and short plants of each container were exactly opposed to each other. This process of selection, instead of random selection from the whole population of fifty-six plants, ensured that differences between the paired plants of a container which were linked to the property (height) used to assign them to their respective batches would remain free to exert their effect and, even if the procedure reversed these differences at successive containers, the asymmetry in the design of the experiment (seven containers to a group) still left a residual unbalanced effect.

It is not surprising, therefore, that the initial plants A and B were not identical, for one batch (A) absorbed during the preliminary period more *total bromide* in its roots and shoots than the other (B). The initial plants represent the base line upon which the effect of the subsequent treatment must be assessed. The experiment consisted of a further period (2 hours) of absorption during which, in one case (three batches), the shoots were ringed and in the light, in the other (three batches), the plants were not ringed.

In the *shoots* there was no difference between the bromide which the ringed and normal plants contained at the end of the experiment, but the ringed plants had less bromide (mean of triplicate batches) in the roots than the normal plants. It is the difference in the bromide content of the roots which has been construed as an effect of a ring upon absorption which became evident *after as short a period as two hours*. In their roots the ringed plants (mean of triplicates) had less bromide than the initial plants (mean of duplicates), whereas the normal plants seemed to have more. Taking the data as Phillis and Mason give them *neither of these differences are significant*—Ringed /Initial P between 0·4 and 0·5; Normal/Initial P between 0·2 and 0·1. In other words, with the evident variability between parallel samples shown by the initial plants, neither the apparent gain in the bromide content of their roots by the unringed plants nor the apparent loss by the ringed ones is real. The difference as between Normal and Ringed groups is open to the inter-

pretation that it was inherent in the plants *before they were so treated and resulted from the way in which they were grown and selected for experiment*, for in each batch of seven plants there was an excess of either the less or the more well-grown shoots. Whether the apparent difference between the ringed and unringed plants did or did not follow from the orderly manner in which they were selected for treatment, it is evident that the undoubtedly significant difference between the roots of the final samples (Normal/Ringed $P < 0.01$) *is no greater than that which already existed between the duplicate initial samples.* Recognizing that as prepared for the experiment the plants of each group fell into two batches (A and B or Normal and Ringed) which were not comparable but differed in respect of properties linked to the height of the shoot, then one can compare the Normal plants with initial plants A and the Ringed plants with initial plants B and show that during the two-hour period of the experiment the *bromide contained in the root did not change.* Thus the first conclusion of Phillis and Mason does not stand.

The gain of bromide by the Normal and Ringed plants in their shoots was large and is not obscured by the difference present in the duplicate initial samples. It is, moreover, consistent with other data that bromide should freely pass a ring. It follows from the experiment that bromide was absorbed during the two hours when the plants were in the light and passed to the shoot without any significant effect of the ring. Since the amount in the root did not change significantly, the effect is as if *all* the bromide absorbed during this period was absorbed by the shoot. The marked difference in the rate of absorption and transfer to the shoot between the plants during 17 hours of darkness (19 mg. per 7 shoots) and the subsequent 2 hours in the light (37·7 mg. per 7 shoots) is a difference *in uptake by the shoot* due to the resultant of all those effects which follow when the shoot is in the light.

Phillis and Mason also conclude that increased *transpiration* increased the uptake of bromide by cotton plants. This conclusion is not established, for the reason that after growth in culture solution the test cotton plants absorbed in darkness during 16 hours a total of 43·6 mg. of bromide in their shoots and 41·3 mg. in their roots. A parallel series was exposed to light and open conditions during 9 hours after 7 hours' contact with bromide in the dark; these contained 76 mg. of bromide in their shoots (mean of three) and 41·1 mg. in their roots. The difference, which was all in the shoot, was a difference due to a *shoot in the light* during 7 hours compared with one in the dark. The growth of 7-weeks-old cotton plants is rapid and it would be quite conceivable that the shoots (especially the leaves) in the light would grow more than those in the dark—in fact it would be surprising if this effect were not at least equal in magnitude to that shown by the intake of bromide by the shoot (ratio of 76 : 43). On the other hand, the effect of the treatment on water loss was great and out of all proportion (ratio of 96 : 10) to the effect on transfer of bromide to the shoot with which it is compared. There is every reason to believe that, of all the varied effects which may accrue from placing a shoot in the light (high concentration of respiratory substrates, oxygen-carbon dioxide

relations favourable to respiration, greater transpiration, increased enlargement of the laminae, &c.), it is the effect of light on the growth of the shoot which is the operative one and which determines the salt intake of plants which, previous to experiment, were exposed to full salt supply. The effect, therefore, which is ascribed to transpiration *per se* may really have been due to the fact that under the conditions which produced this difference one set of shoots developed more than the other. Because the experiments lasted for hours instead of days is no reason for assuming that the processes of growth play no part.

In their fourth paragraph of conclusions Phillis and Mason are concerned with 'presumptions'. First they presume that transpiration affects uptake by altering the concentration in the absorbing regions of the root. The only data they give show that despite the observed difference in transpiration and transfer to the shoot the *total amount* present in the root remained the same. If the concentrations were in fact different, this must have been due to some unspecified difference (e.g. size of roots) which was ignored elsewhere in the interpretation. They also *presume* that in some unspecified way transpiration 'oxygenates' the root. Such a role might with some justice be ascribed to light via photosynthesis but not directly to transpiration. It is also *presumed* that assimilation affects the 'solvent capacity' of the root. Solvent capacity is itself hypothetical; when it becomes tangible by measurement of actual concentrations in tissue fluids it seems to be the familiar property of accumulation in cells in a new but indeterminate form. However, the effects which Phillis and Mason actually described as a consequence of their treatment concerned *only total amounts* and were evident in the shoot and not in the root.

Beyond the evident fact that bromide can freely pass a ring—a result frequently encountered since 1930 in the investigations referred to—*all* the conclusions at which Phillis and Mason arrive in their paper seem to be mistaken or unsupported by the facts put forward; they increase, rather than dispel, the existing confusion in the literature of the subject. Final judgement should be reserved and await the fuller investigation and more critical interpretation which is required.

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Studies in Germination and Seedling Growth

I. The Water Content, Gaseous Exchange, and Dry Weight of attached and isolated Embryos of Barley

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With seven Figures in the Text

I. INTRODUCTION

IT has been known for some time that the conditions to which the seed is exposed during germination affect not only the course of that process but also the whole subsequent growth and development of the plant (Kidd and West, 1918, 1918a, 1919). The significance of the subject has recently been emphasized by an extensive series of observations on the effect of low temperatures during germination on the growth of cereals (Gassner, 1918; Purvis, 1934). Clearly the particular effects of the conditions of germination on the growth and metabolism of the seedling during this phase of development are of primary importance.

The elucidation of the nature of the effects on the seedling of conditions applied to the seed is, however, complicated by the fact that the environment to which the embryo is exposed is not necessarily that which is external to the seed. Thus the investigation of the effects of different conditions on growth is facilitated if the embryo can be cultured independently of the seed. This consideration has determined the choice of barley as the material for investigation. Several investigators have shown that isolated embryos of barley and other

cereals can be cultured successfully (Brown and Morris, 1890; James and James, 1940). Excision, however, modifies considerably the subsequent growth of the gramineous seedling (Schander, 1934, and De Ropp, 1939). For a satisfactory study of isolated embryos for the elucidation of the influence of external factors on the seed a knowledge is therefore required of the change in environment involved in excision and also of the effect of this change on the general metabolism. These two aspects of excision are the subject of the present series of observations.

A comparison of the water content and the gaseous exchange of isolated and attached embryos provides some indication of the incident levels of water, oxygen, and carbon dioxide inside and outside the seed. The environmental change undoubtedly involves factors other than these, but together with the nutritional factor they are of primary importance. Data on the nutritional factor are available from the work of other investigators. Water, the gaseous exchange, and dry weight changes are important features, and a comparison of these in attached and isolated embryos distinguishes certain immediate, albeit limited, effects of the different environments on metabolism.

The investigation is, for two reasons, restricted to the first 12 hours of development. Another set of observations suggests that the subsequent dry weight increment of the isolated seedling is powerfully affected by the length of the period of attachment during the first 12 hours of growth; and observations on water content, respiration, and dry weight made during this phase can be interpreted independently of the nutritional factor, since Brown and Morris (1890) and James and James (1940) have shown that the mobilization of at least insoluble carbohydrate reserves does not begin until about 24 hours after the seeds are brought into contact with water.

II. MATERIALS AND METHODS

As the nutritional level within the seed during the first 12 hours is low, the most satisfactory medium to which the seedling can be transferred after excision is clearly water; but excision induces a more rapid uptake of water and oxygen by the seedling, which in turn promotes a higher respiration rate. The isolated embryo may therefore risk a starvation from which the attached seedling is protected by the low availability of water and oxygen. Thus it is necessary for the purpose of studying the metabolic effects that the reactions of the isolated seedling should be observed both in water and in a culture medium. Accordingly two series of experiments with isolated embryos are included in the present series, one in which the embryos are transferred to water after excision, and another in which they are transferred to a culture solution. The culture medium used throughout these experiments consists of mineral salts dissolved in 2 per cent. sucrose. The proportions and nature of the mineral constituents are based on a complete nutrient mixture developed by Gregory and Richards (1929) for use with barley.

Each series of isolated embryos in this investigation comprises several groups, each group being excised at a definite time after water uptake by the

whole seed begins. The excisions for each group are made at intervals of 2 hours. The purpose of this experimental variable is to determine the effect of the period of attachment to the parent seed on the water content, the gaseous exchange, and the dry weight established after excision. As indicated above, the final growth of the seedling varies according to the time of excision after germination has begun. This suggests the possibility that the relations studied in the present series of observations are also affected by the time of excision. The incidence of such effects must clearly be of considerable significance for the interpretation of the consequences of excision in terms of the environmental change that the treatment occasions.

The experimental procedure involves inducing germination in the whole seed by promoting water uptake, and then transferring the seedling after excision to an artificial medium. During the preliminary period of water uptake the conditions of water availability must be constant. This condition is difficult to achieve with solid media. Intimate contact between the seed and the medium is not uniformly established and the area of contact varies. These objections are avoided by establishing uptake from pure water. Complete immersion in water is, however, unsatisfactory since the gaseous exchange of the seed thus may be highly restricted. Accordingly, the material is floated on the liquid on muslin secured to a closed glass ring made with very thin-walled glass tubing. In these circumstances the conditions for uptake do not vary and the gaseous exchange is not as restricted as with complete immersion. For purposes of comparison it is clearly desirable that the embryos should be exposed to the same conditions as those for the whole seed. Accordingly after excision the embryos are floated on liquid media—either water or culture solution—by the method used for entire seeds.

Since the experiments last only 12 hours no special precautions are required to ensure sterility; this simplifies considerably the technique for isolating the embryos. After removing the pales an incision is made in the seed coat along the edge of the scutellum. The embryo is then dislodged by inserting the tip of a scalpel between the scutellum and the endosperm and levering it away from the parent seed. With material that has been soaked for 2 hours or more this technique yields satisfactory results, the embryos being removed without injury and without any adhering endosperm fragment. The method is inadequate with dry seeds and no technique has been devised for this condition; data on the reactions of embryos isolated before germination begins have therefore not been obtained.

The water uptake and dry weight data are from samples removed from a larger population. They are weighed immediately, and then again after heating for 24 hours in an oven kept at 80° C. If the material is isolated embryos which have been exposed to a free liquid, the surface liquid is removed by pressing them between two layers of filter-paper.

A Barcroft respirometer is used for the measurement of the gaseous exchange, the technique having been adapted from the directions given by Dixon (1934). The carbon dioxide production and oxygen uptake determina-

tions are made on separate samples in separate respirometer units. The errors of the method, particularly those involved in determining the rate of carbon dioxide production, are high; they are due largely to the peculiar nature of the material and are discussed later.

All observations other than the gaseous exchange measurements are made in a chamber in which the temperature is controlled at $22°$ C.$\pm 1°$ C. The temperature of the bath in which the respirometer flasks are immersed is also controlled at the same temperature, with a fluctuation of $\pm 0\cdot 1°$ C.

The individual samples in a series of observations each comprise 20 seedlings. Occasionally they are larger, but the size is limited by the necessity that the isolations made every 2 hours should not be spread over a considerable proportion of that interval. A group of 20 embryos can be excised within 15 minutes. The dry weights of different samples of 20 embryos determined immediately after excision do not differ by more than 6 per cent.

In the next section the primary data are given in graphical form, the data for the isolated embryo being presented along with the corresponding data for the attached embryo, i.e. in the entire seed. The curves for isolated embryos are extrapolated back to points on the relevant curves for entire seed corresponding to the excision times. This arrangement is clearly appropriate with water content and dry weight; the considerations that justify it also with the gaseous exchange are discussed in the next section.

III. EXPERIMENTAL RESULTS

i. *Water uptake by the embryo.*

Figs. 1*a* and 1*b* show the water content at 2-hour intervals of embryos taken from seeds floated on water, the same data for attached embryos (entire seeds) being repeated in Figs. 1*a* and 1*b*. This figure also shows the effect on water uptake of transferring the embryo from the seed to water (Fig. 1*a*) and to the culture solution (Fig. 1*b*).

In the seed the rate of water uptake by the embryo is evidently low (Fig. 1, curve *E*), the rate being relatively high between 2 and 6 hours but very low between 6 and 12 hours; this suggests the development at this stage of a temporary equilibrium. When the embryos are transferred either to water or to a culture solution, there is a first phase of rapid uptake leading to an equilibrium state, established at about 4 hours, in which the rate is low, followed by second phase (in those series excised early enough to show it) of rapid uptake. The rates in the first and second phases are unaffected by the character of the medium, water or culture solution, to which the embryos are transferred. The water contents at 2, 4, and 6 hours after excision are approximately the same with both media. In the second phase only is the effect of the medium apparent; at this stage the water content in a culture solution increases more rapidly than in water.

The effect of time of excision can only be assessed by comparing corre-
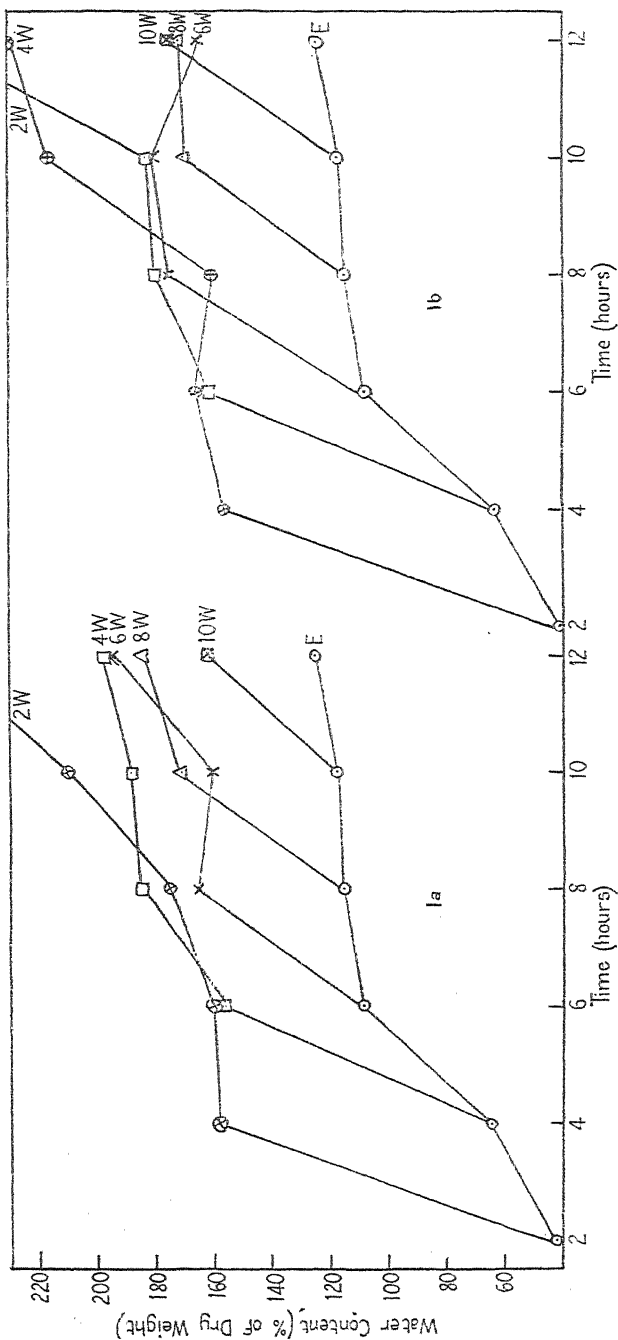
FIGS. 1a and 1b. Fig. 1a water content of attached E and isolated embryos on water. Fig. 1b the same on culture solution. Numbers attached to curves indicate time of excision (hours) after water uptake by whole seed has begun.

sponding periods after excision. Such comparisons show little or no differences that can be attributed to this experimental variable.

ii. *Gaseous exchange of embryo.*

Fig. 2 shows the hourly rates of oxygen uptake and carbon dioxide production by entire seeds and by isolated embryos excised at 2 hours and transferred to water; in the same figure two curves (from Fig. 1*a*) have been included showing the simultaneous drift in water content in each series. Arbitrary smoothed curves have also been drawn through the series of experimental values for the gaseous exchange of the entire seed. Figs. 3*a* and 3*b* show the hourly rates of oxygen uptake of entire seeds (data from Fig. 2) and of embryos excised at intervals of two hours and transferred to water (3*a*) and culture solution (3*b*). Table I gives the respiratory quotients of each of the several series shown in Figs. 2 and 3. In each of these figures the isolated embryo curves are extrapolated back to points on the appropriate entire seed curves corresponding to the excision times. This treatment is clearly only justified if the respiration of all tissues in the entire seed other than those of the embryo is negligible. The gaseous exchange of the endosperm and associated tissues was determined within the first hour after separation and found to be only 5 per cent. of that of the entire seed. Subsequently it increased slowly, due no doubt to an enhanced water uptake. Barnell (1937) examined the carbon dioxide production of barley grains from which the embryo had been removed 70 hours after germination began, and found it to be sufficiently large to warrant applying a correction to the whole grain data when estimating the respiration rate of the embryo in the seed. The differences between the two sets of results are probably due to different experimental circumstances. The material in this investigation had been germinating for 6 hours, in Barnell's for 70 hours; in this investigation the observations were begun 20 minutes after separation; in Barnell's apparently some hours elapsed.

Table I

Respiratory Quotients of the several experimental Series shown in Figs. 2 and 3

Hrs. in col. 1 refer to time from beginning of water uptake by whole grain. *A* indicates attached embryo, *i* isolated embryo; *W* embryos transferred to water, *C* embryos transferred to culture solution. The numbers refer to the time of excision (hrs.) after beginning of water uptake by whole grain.

Time (hrs.)	*A*	*2iW*	*2iC*	*4iW*	*4iC*	*6iW*	*6iC*	*8iW*	*8iC*
3·5	1·18	0·80	0·81						
4·5	1·12	0·96	0·95						
5·5	3·38	0·97	1·03	0·84	0·86				
6·5	2·27	0·97	1·11	0·98	0·97				
7·5	1·73	1·03	1·14	1·06	1·09	0·91	0·88		
8·5	3·63	1·00	1·20	1·09	1·14	1·05	1·04		
9·5	2·66	1·04	1·20	1·09	1·17	1·13	1·17	1·01	0·97
10·5	2·65	1·00	1·23	1·12	1·26	1·15	1·25	1·01	1·07
11·5	2·60	1·01	1·25	1·08	1·27	1·12	1·23	1·3	1·13
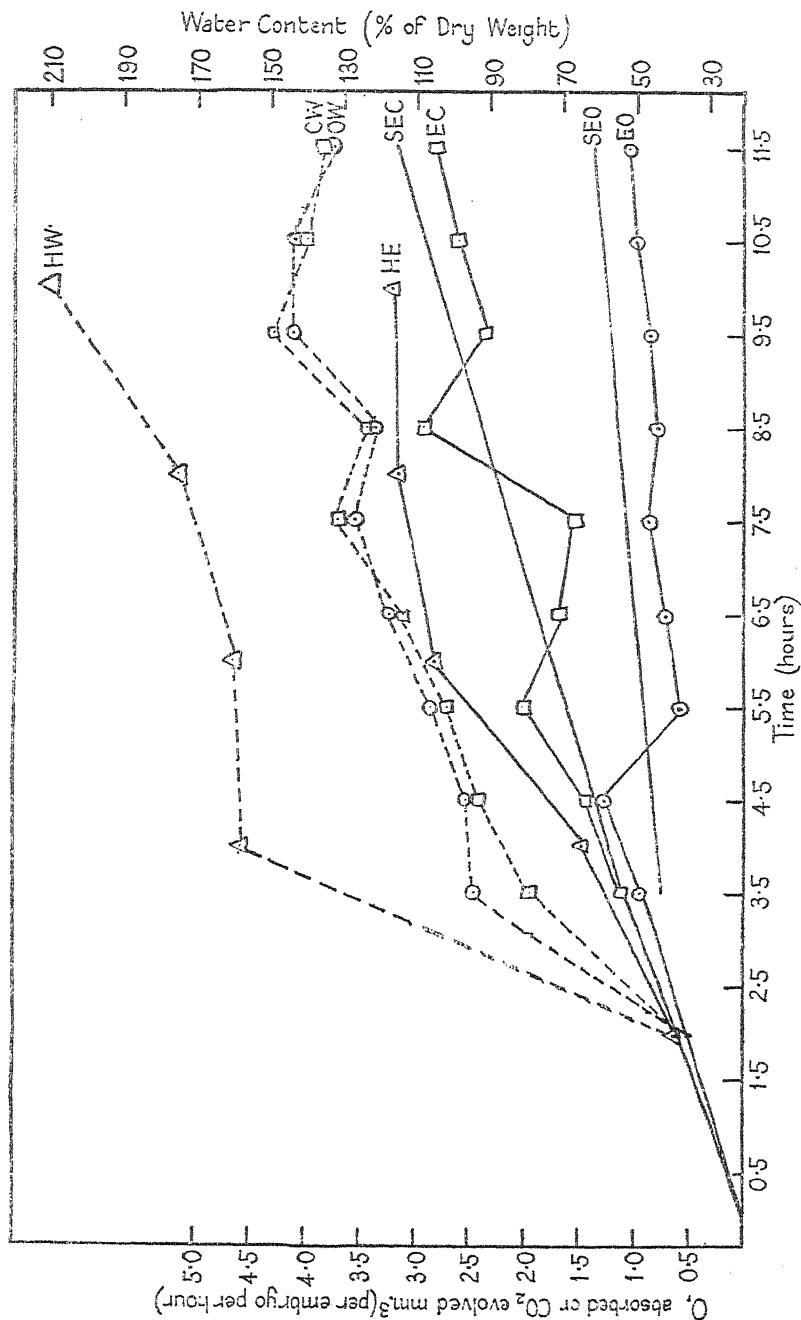
FIG. 2. Water content *H*, rates of carbon dioxide emission *C*, and oxygen absorption *O*, of isolated (*W*) and attached embryos (*E*). *S* indicates smoothed curve through experimental points. Embryos excised at 2 hours. Isolated embryos and entire seeds on water.
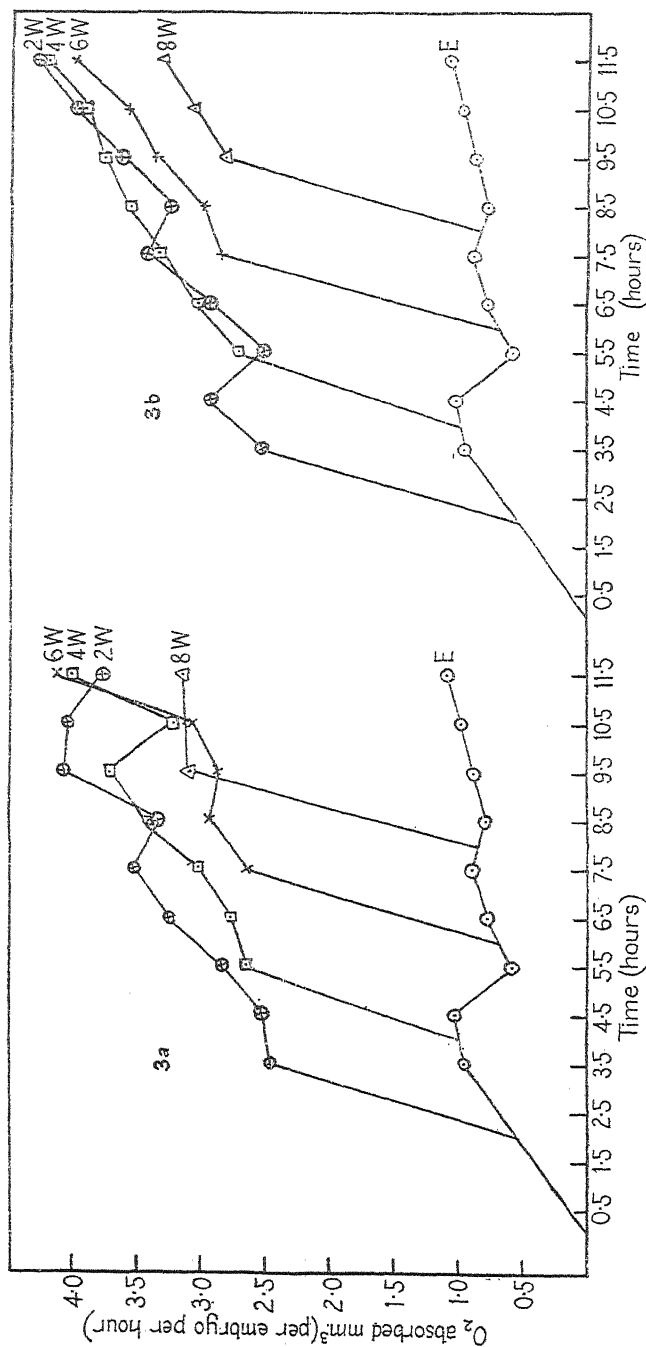
FIGS. 3*a* and 3*b*. Oxygen uptake of isolated (*W*) and attached (*E*) embryos. Isolated embryos on water (3*a*) and on culture solution (3*b*). Entire seeds on water. Numbers attached to curves indicate time of excision (hours) after water uptake by whole seed begins.

It is evident from Fig. 2 that with entire seeds both rates of the exchange fluctuate considerably. There is no reason to think that this is due to corresponding fluctuations in the experimental conditions, and it is apparently a characteristic of the material itself. In the original experiments manometer readings were taken every 30 minutes, but the values given in Figs. 2 and 3 are calculated from the hourly readings. In Fig. 4 values for the oxygen uptake
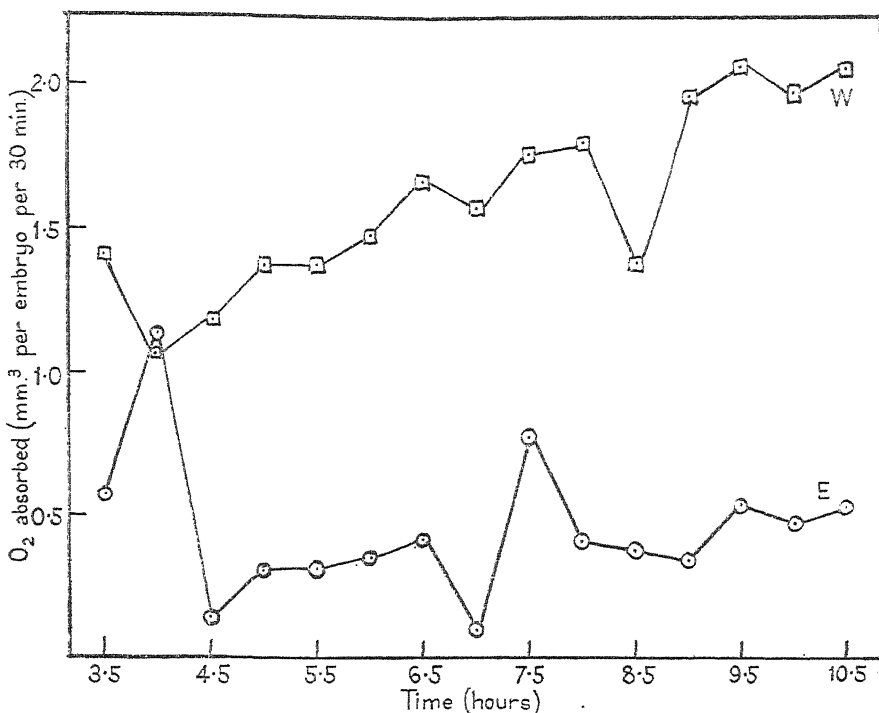
FIG. 4. Oxygen uptake by isolated (*W*) and attached embryos (*E*). Embryos and seeds in water. Embryos excised at 2 hours.

for entire seeds and embryos isolated at 2 hours and transferred to water are given which are calculated from the successive 30-minute readings. The fluctuation over 30-minute intervals is clearly greater than it is over hourly intervals. Since the oxygen uptake of the isolated embryo also fluctuates it would seem probable that this is the origin of the comparable drift in the oxygen uptake of the whole seed. The fluctuations in the rates of oxygen uptake place a serious limitation on the calculated values for carbon dioxide production. With the method used in these experiments oxygen absorption is measured directly, but carbon dioxide production is calculated from a pressure difference due to the unequal exchange of the two reactant gases determined on another sample. Since fluctuations in different samples may not coincide the error involved in the estimation of the carbon dioxide values may be fairly high. Thus, whereas the oxygen data are reasonably accurate the carbon

dioxide values are probably not so when taken over short intervals of time. Over larger intervals, of course, they acquire greater significance.

Notwithstanding the large fluctuations in the rates of the exchange for the whole seed (Fig. 2), certain trends can be discerned. Whereas the rate of carbon dioxide emission tends to increase, that of oxygen uptake tends to remain constant. This difference is emphasized by the smoothed curves drawn through each set of experimental points, and by the respiratory quotient values, which tend to increase with time (Table I, col. *A*).

When the embryo is detached from the seed (Fig. 2) the rates of the exchange increase. Two phases in the drift of the rates with time after excision may be distinguished. The first phase is complete when the first measurement after excision is made. It is characterized by very rapid increases in both rates, by which the rate of oxygen uptake is trebled and that of carbon dioxide emission is about doubled over the rates for the whole seed. In the second phase both rates continue to increase but much more slowly than in the first phase.

The respiration rate of the embryo is apparently only very slightly affected by the nutrient. There are only slight differences between the values for corresponding series given in Figs. 3*a* and 3*b*. It would seem that oxygen uptake is only slightly affected by the nutrient medium. The data of Table I suggest a slightly greater rate of carbon dioxide production when the nutrient is present. In three out of the four relevant series the respiratory quotient is greater with the culture medium than it is with water. The difference is only small and does not indicate any considerable nutrient medium effect. The effect of time of excision is not pronounced. There is some indication, however, that the rate of oxygen uptake established immediately after excision tends to increase as excision is postponed.

In seven of the eight isolated embryo series (Table I) the respiratory quotient is at first less than one; subsequently it rises gradually until it attains at least in water an approximately steady value.

iii. *Dry weight changes in embryo.*

Figs. 5*a* and 5*b* show the dry weights of attached and of isolated embryos excised at 2, 4, 6, and 8 hours and transferred to water (5*a*) or to a nutrient medium (5*b*). The same data for attached embryos are shown in both Figs. 5*a* and 5*b*. The dry weight change with time for attached embryos shows no consistent trend. The dry weight of the isolated embryo decreases when it is transferred to a fluid medium. Here again two phases characterize the general drift. During the first 2 hours there is a sharp and pronounced fall; subsequently, although the decrease continues, it is less precipitate.

There is a notable difference between the two sets of data. The dry weights of embryos in culture solution are consistently lower than those in water.

There is some indication that the initial fall in dry weight is greatest in the series excised at 2 hours; this is certainly the case for those in water. In culture

solution the difference between this and the other three series is, in this respect, less pronounced.

IV. DISCUSSION OF RESULTS

As indicated in the first two sections relating to water content, gaseous exchange, and dry weight, three separate topics are involved in the subject of
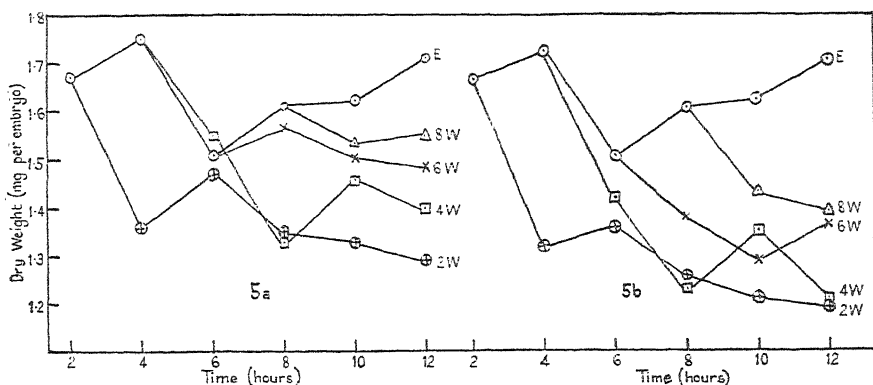
FIGS. 5a and 5b. Dry weight of isolated (*W*) and attached embryos (*E*). Isolated embryos on water (5a), culture solution (5b). Numbers attached to curves indicate time of excision (hours) after water uptake by whole seed begins.

the present investigation: (i) the general effect of excision, (ii) the effect of the nutrient level of the culture medium, and (iii) the effect of the time of excision.

i. *The general effects of excision.*

After excision the water contents and the rates of the gaseous exchange increase, while the dry weight falls. Thus the data for the isolated embryo are sharply distinguished from those for the attached. But the changes in each of the relations with time after excision fall, as shown in the last section, into two phases. The general array of data therefore comprises three groups, the data for the attached embryo, those for the isolated embryo immediately after excision, and the final data for the isolated embryo. The first phase after excision is completed rapidly and suggests the attainment of a new equilibrium condition consequent upon excision. The operation of predominantly physical factors during the first phase is also suggested by certain other considerations presented in the sequel. This state of affairs is clearly of considerable significance for the interpretation of the observations on attached embryos. Accordingly the isolated embryo data of the first phase are discussed along with those for the attached embryo, the isolated embryo data of the second phase being treated separately.

(*a*) *Development of attached and of isolated embryos immediately after excision.* The barley grain may be considered as a system of an endosperm and an embryo both enclosed within a seed coat. Thus when in the seed, the reactions of the embryo in terms of water content, gaseous exchange, and dry weight must be considered in relation to the conditions established by the

endosperm and seed coat. Excision involves a removal of the conditions which these two impose. Data are available from the work of other investigators from which the nature of these conditions may be assessed. Certain additional observations relevant to this theme made by the present investigator are given here, since their purpose is that of elucidating the significance of the primary data.

After excision the water content increases rapidly and reaches the same equilibrium value in both water and culture solution; this indicates that in this stage of absorption osmotic forces are not involved and that consequently water uptake is not being conditioned by the supply of oxygen. The embryo may therefore be considered as a lyophilic colloidal mass in which the equilibrium water content is proportional to the level of availability. It is evident that the level inside the seed is considerably lower than it is on a free water surface, the properties of the seed coat and the endosperm being such as to restrict the supply of water to the embryo. Schroeder (1911), Collins (1918), Braun (1924), Brown (1932), and Tharp (1936) found cutinized layers in the enveloping membranes of various Gramineae. Being cutinized these layers, if they do not entirely prevent general surface absorption by the seed, must restrict the permeability of the seed coat to water. Experimental evidence for this conclusion is available. Shull (1913) compared the rate at which the equilibrium water content is reached in Xanthium and cereal grains when the seeds are immersed in water. With Xanthium this state is reached within 24 hours, whereas with cereal grains not until the seventh day; from which he concluded that resistance to the diffusion of water must be high in cereal and low in Xanthium seed coats. Brauner (1928) examined the permeability to water of isolated cereal seed coats and confirmed the earlier conclusion of Shull. The permeability of the seed coat to water is therefore low, and this condition is undoubtedly the origin, at least in part, of the low level of water availability to the embryo in the seed.

The influence of the endosperm on the water uptake by the attached embryo depends on the relative suction pressures of the two tissues. A measure of this relation is provided by a comparison of the equilibrium water content after exposure to atmospheres of varying relative humidity. Endosperms and embryos suspended over sulphuric acid of varying concentration and over water for 48 hours give the values shown in Fig. 6. The water content continues to increase for some days after this period, but the further increase is small and the values obtained at 48 hours may be considered as those of the equilibrium state. It is evident that the water content of the endosperm is higher than that of the embryo in low, but lower in high, relative humidities; this indicates that with increasing water content the water-absorbing capacity of the endosperm falls much more rapidly than does that of the embryo. Thus when, as in the seed, these two tissues are in competition for water, then the rate of absorption by the embryo relative to that of the endosperm should become increasingly greater with time. That such does in fact happen during the first 6 to 8 hours of overall absorption by the seed is shown by the data of Fig. 7; the seeds for this series of determinations were

floated on water, samples were taken at intervals of 2 hours, and the water content of the embryo and endosperm determined separately.

It is evident that the comparatively high rate of absorption of the embryo persists only until about the 6-hour stage (Fig. 1). Subsequently the rate of
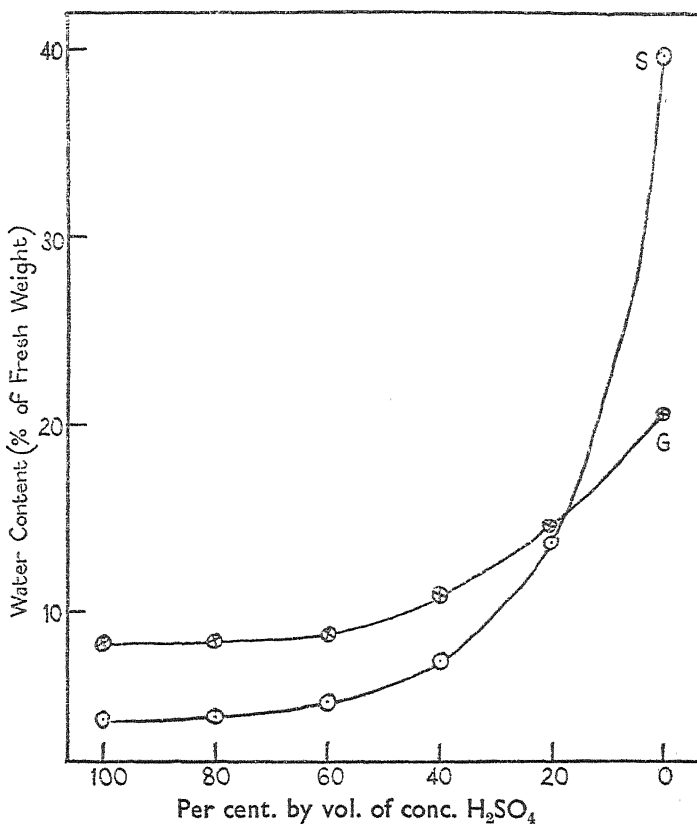
FIG. 6. Equilibrium water contents of endosperms (G) and embryos (S), after exposure to atmospheres of varying relative humidity.

uptake increases; but it does so at a time when excision still promotes an enhanced uptake, and when the water content of the seed is still increasing. Clearly the decreasing rate of absorption after 6 hours cannot be due to an approach to the saturation water content or to a restricted water supply within the seed. It might, however, be due to a mechanical restriction to further expansion by the embryo. The seed in the dry state has a wrinkled surface, particularly in the region of the embryo. When water is absorbed the contents of the seed swell with the immediate effect of filling out the folds. This stage is reached about 4 hours after water absorption begins; further swelling does not apparently cause a break in the seed coat, for dyes do not penetrate into the endosperm until about 30 hours later. The swelling occurring between 4 and 36 hours must therefore induce a progressive stretching of the enveloping

membranes, and the internal equilibrium apparently established between 6 and 10 hours may be the effect of a balance between the mechanical pressure exerted by the swelling gel, and the resistance of the seed coat to further elastic
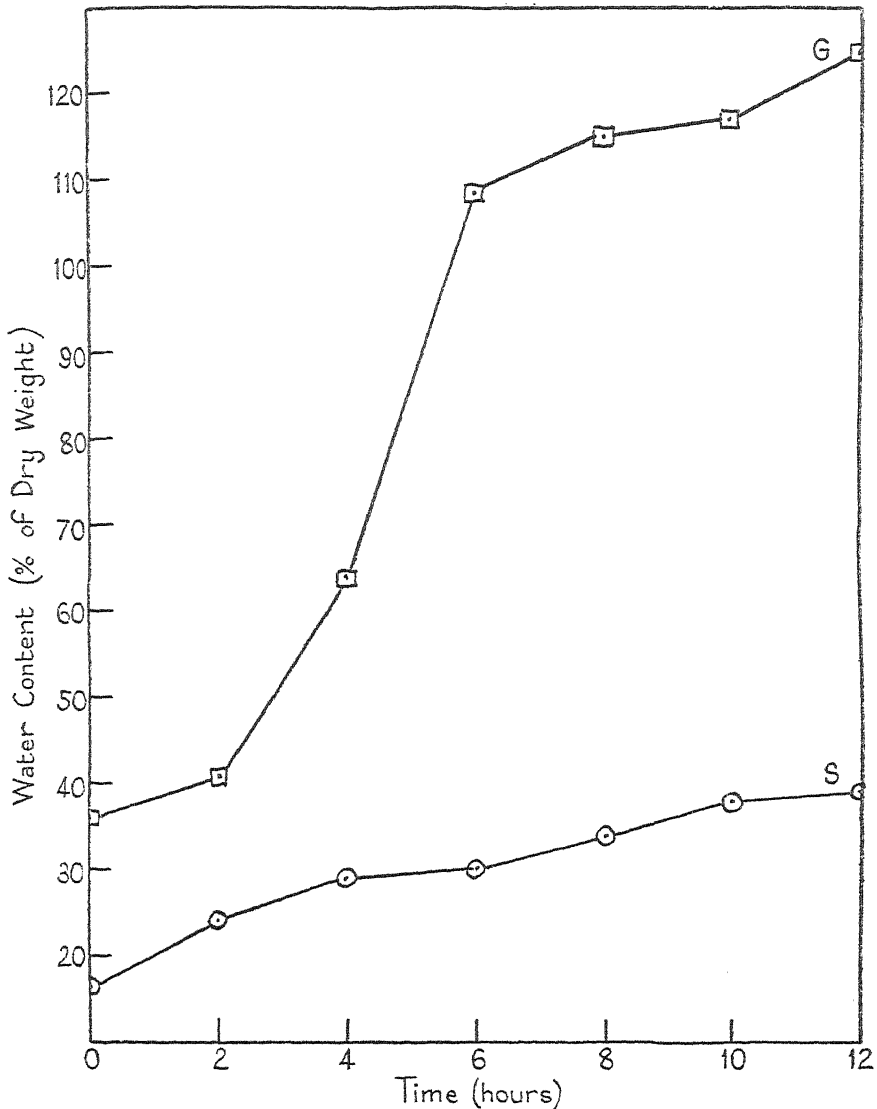
FIG. 7. Water content of endosperm (*G*) and embryo (*S*) established in intact seeds floated on water.

extension. Harrington and Crocker (1920), working with Johnson Grass, found that failure to germinate is on occasion due to membranes which prevent the swelling of the seed and thus limit the absorption of water.

The respiration data for the whole seed of Fig. 2 are in some respects

strikingly different from those recorded by James and James (1940). These workers found that with whole seeds both rates of the exchange increased from 0 to 10 hours, and whereas the rate of carbon dioxide emission increased steadily, that of oxygen uptake fluctuated. The time drift of the R.Q. rose from a low value for the dormant grain, passed through a maximum (about 1·5), and then declined to a new low level at about 12 hours, the peak value of the R.Q. at about 4 hours being due to an oxygen lag and not to a 'sudden release of accumulated carbon dioxide'. The differences between the two sets of data are undoubtedly due to different experimental conditions. In the experiments of James and James the paleae were removed from the seed, in the present experiments they were not; in their experiments the seeds were cultured on sand moistened with a full culture solution, in these they were floated on a free water surface and later transferred to a Barcroft respirometer in which they were immersed in water. James and James state that 'the origin of the oxygen block seems to be physical rather than chemical and to be associated with the wetting of the seed. The low permeability of water to oxygen must materially increase the diffusion resistance if a film of water is placed between the respiring cells and the surrounding air. This condition is exaggerated if the grains are immersed in a shallow dish of water instead of being pressed into moist sand.' The conditions in which the seeds were maintained in the present series of experiments approximate to those of the shallow dish of water, and it is probable that the water content of the enveloping membranes was very much higher than in the experiments of James and James, the comparatively low water content of the endosperm notwithstanding. The cutinized membranes, which, as indicated above, restrict the supply of water to the endosperm, develop over a layer of cells immediately outside the aleurone layer (Tharp, 1936). Their position is such as to affect the water content of the endosperm but not that of the bulk of the seed coat. Contact with free water would therefore lead to the establishment of a continuous film of water round the seed, which would moreover remain undisturbed whatever the agitation in the liquid outside the seed, the stability of the film being sustained by the system of rigid cell walls within which the water is enclosed. Thus a restrictive effect on the diffusion of oxygen is inevitable even when the whole mass is being vigorously shaken as it is in the Barcroft apparatus.

The effect of excision on the rates of the exchange is similar to that on water uptake. There is an initial phase of rapid increase which is complete within 2 hours (Figs. 2 and 3). Since excision involves separation from the other tissues of the seed, the low rates must be related to the properties of these tissues, and the enhanced rates after excision to the removal of the conditions imposed by them.

Reasons have already been advanced for assuming that the diffusion resistance to oxygen in the seed coats of these experiments is high. Experimental evidence provided by a number of investigators shows that the seed coats of many seeds are in fact highly impermeable to oxygen (Brown, 1942).

The comparatively low rate of oxygen uptake in the seed must therefore be due at least in part to the low permeability of the seed coat for oxygen.

Excision by removing the influence of the seed coat increases the supply of oxygen to the embryo. It is, however, improbable that the subsequent higher rate of absorption is due to this factor alone. Fig. 2 shows the simultaneous changes in the rates of the gaseous exchange and in the water content of attached and isolated embryos. The general similarity between the curves for carbon dioxide emission and water content is striking. A similar correspondence in entire seeds of rye has been recorded by Pringsheim (1932). With the entire seed although the rate of oxygen absorption remains approximately constant the rate of carbon dioxide emission continues to increase. Since these trends are maintained throughout the period of the experiment it may be taken that they indicate corresponding trends in the exchange of the embryo. Now since the rate of oxygen absorption does not change, the increased carbon dioxide production cannot be due to an enhanced supply of oxygen; but it may be due either to a changing respiratory mechanism which is increasing the respiratory capacity of the seedling independently of the environment, or to the effect of a changing environmental factor other than oxygen availability. The condition represented by the first alternative implies that the higher rate of carbon dioxide production also involves a potentially higher rate of oxygen absorption, which should become apparent after excision. In fact, the rate of uptake immediately after excision is apparently greater when the embryo is excised at 8 hours than it is when it is excised at 2 hours after water uptake begins. This difference is, however, relatively much smaller than the corresponding increase in the rate of CO_2 production of the attached embryo, which is therefore probably due predominantly to the operation of some environmental factor. Although oxygen absorption remains constant, water content increases. Bailey and Gurjar (1918) have measured the respiration of wheat grains maintained at known water contents and they have shown that the rate of carbon dioxide production increases with increasing water content, the increase being greatest for water contents above 17 per cent. The present investigator (Brown, 1942) has shown that the gaseous exchange of the isolated cotyledon may be conditioned by the level of water availability. Thus it would seem probable that the basis for the correspondence between the curves for water content and carbon dioxide emission is an effect of the water content on the uptake of oxygen.

The determination of the partial pressure of oxygen with which the contents of the seed is in equilibrium depends on the considerations presented above. Since in the earlier stages of germination the respiration rate is not being conditioned primarily by internal changes in the respiratory apparatus, the time factor can be disregarded and comparisons may be made on the basis of water content, which is probably the most important limiting factor other than oxygen availability. The value required is thus given by a comparison between the uptake of the isolated and of the attached embryo at the same water content, the estimation being based on the assumption that oxygen

uptake is directly proportional to the partial pressure. All the values for water content of the embryo of the whole seed correspond only with values on the extrapolated portion of the curve of uptake for isolated embryos, and the comparable oxygen uptake values of these are also all on extrapolated portions. Thus although it is possible to match all the oxygen absorption rates of the attached with comparable values for the isolated embryo, the comparison involves values from a pair of extrapolated curves which must be liable to considerable error. The extrapolated curves, however, establish a general order of values which is probably sufficiently accurate for the present purpose. The oxygen uptake rates for the whole seed are clearly more satisfactory if they describe a trend; they are therefore taken from the smoothed and not from the experimental curve. The values calculated in the manner described above for water contents of 105, 90, and 70 per cent. are 12·1, 10·2, and 9 in terms of percentage composition of one atmosphere, from which it is evident that the partial pressure of oxygen with which, in the conditions of these experiments, the contents of the seed are in equilibrium is about 0·1 of an atmosphere.

The corresponding condition with carbon dioxide is a function of the rate at which the gas is produced by the embryo, of the permeability of the membrane to the gas, and of the external concentration in the atmosphere. The external concentration can be taken as zero and the internal rate of production as that of the external rate of emission. No data are available on the permeability of the barley seed-coat membrane to carbon dioxide, but the structural features which determine the permeability to water and therefore probably to gases are not unlike those of the inner seed coat membrane of Cucurbita. With the Cucurbita seed coat the author (Brown 1940) found that the relative permeabilities to carbon dioxide and oxygen are in the ratio of 4 : 1. If this is assumed for barley, then with an R.Q. of unity and with a percentage oxygen concentration difference of 10 per cent. across the membrane, the internal carbon dioxide concentration should reach about 2·5 per cent. With an R.Q. of about 3, however, the corresponding carbon dioxide concentration becomes about 7·5. It may, therefore, be assumed that the partial pressure of CO_2 with which the content of the seed is in equilibrium corresponds to 0·1 of an atmosphere.

No precision can be attached to the estimated values given above since they involve assumptions which, although reasonable, are not derived from immediate experimental evidence; also several important factors of the situation, such as possible changes in the properties of the seed coat membranes have been disregarded. Moreover, the values for the partial pressures given above represent average conditions, but the fluctuations that occur in the rates of oxygen absorption and carbon dioxide production must be accompanied by corresponding changes in the internal concentrations of the reactant gases. It is, therefore, probable that the actual concentrations are at times higher and lower than the average values.

The attached embryo shows no consistent changes in dry weight, but when the embryo after excision is transferred to a liquid medium the dry weight falls

precipitately. No such immediate decrease occurs when the embryos are suspended in a saturated atmosphere. Clearly the effect is related to the presence of the liquid. The decrease is at least twenty times as great as any change that could be caused by respiration, and may be attributed at least in part to a leaching of solutes from the tissues of the embryo.

(b) *Development of isolated embryos during second phase after excision.* The immediate effects of excision are predominantly of a physical character determined primarily by the change in environment. In the second phase, however, the changes are not due to modifications of the environment. Unlike the environment of the seed, that of the artificial medium remains constant. Thus, the changes that occur after the immediate adjustment to excision are due mainly to modifications in the metabolic condition of the seedling.

Six hours after the excisions are made there is a second rise in water content (Fig. 1). Since the imbibitional capacities of the lyophilic colloids have been satisfied at an earlier stage, this suggests the development of osmotic absorption and the induction of an active phase of seedling growth. There is no such break in the oxygen uptake time curve (Fig. 3). Immediately after the initial phase of rapid uptake the rate continues to rise slowly throughout the period of the experiment, which since it occurs even in water, indicates a steadily increasing elaboration of the respiratory mechanism.

During this second phase the dry weight continues to decrease. Although the decrease in weight is still greater than any reduction that could be attributed to respiration, the increasing intensity of this process in this phase must contribute substantially to the observed effect. The relative stability of the dry weight of the attached seedling is also no doubt related to a low respiration rate.

ii. *Effect of the nutrient level in the culture medium.*

The differences between the several sets of isolated embryos in water and in the culture solution are not pronounced. The water content data show only slight differences between the two series. The significance of the similarity between the two series in the first phase of absorption has already been discussed. In the second the approximately similar values must mean that sugar is not absorbed in sufficiently large amounts to affect appreciably the osmotic pressure of the vacuolated cells. The rates of oxygen uptake, and particularly of carbon dioxide production, are slightly higher in the culture solution than in water, but again the difference is not great. The fact that it is higher suggests that at least some of the solutes are being absorbed in small amounts. The most pronounced effect of the culture solution is shown with dry weight, the fall in weight being more intense in the culture solution than it is in water.

iii. *Effect of the time of excision.*

The difference between the series excised at different times is not great. There is some indication that the series excised at 2 hours differs in several respects from those excised subsequently. With this series the rates of the

exchange are low, the respiratory quotient with culture solution is low, and the leaching effect is particularly pronounced, but the dominant features of the course of development within the first 12 hours of growth are apparently unaffected. After excision the seedlings display the same succession of phases such that parallel courses are maintained whatever the time of treatment. The time of treatment does not apparently affect greatly the level at which the readjustment is made in the first phase, nor does it shorten or prolong the succession of phases in the later that are apparent in the earlier series. The later series do not in fact show the final characteristics of the earlier because they have not had time to develop during the period of the experiment. The primary phases of these later series are approximately the same as the corresponding phases in the earlier series, both in intensity and duration. These relationships are particularly well shown by the water content (Fig. 1) and by the respiration data (Figs. 2 and 3), but less markedly so by the dry weight data (Fig. 5).

Throughout the present experimental period excision increases the rates of water uptake and respiration, and the isolated seedling shows accordingly a more rapid development than the attached seedling. Schander (1934) has observed that the removal of the enveloping membranes from the seeds of various Gramineae promotes a more rapid immediate development, which is attributed to an enhanced absorption of water by the embryo. He, however, also found that seedlings from seeds with intact seed coats ultimately sustained the more vigorous growth of the two series. Malhotra (1933), working with maize, compared the growth of isolated seedlings with that of attached seedlings and showed that excision immediately affects the rate of development of the seedling. The results here presented are therefore similar to those of earlier workers. Thus, although the time of excision has little effect on the immediate course of development of the isolated seedling, the earlier this treatment is applied the more advanced is the development of the seedling at any appropriate time after germination begins in the intact seed. The series excised at 2 and 4 hours in Fig. 1 show the induction of osmotic absorption at a time when the later-excised series are still in an earlier phase of development.

The accelerating effect of early excision on development is only temporary. Ultimately the growth of the later overtakes that of the seedlings earlier excised. The origins of this effect are the subject of another investigation.

V. SUMMARY

1. The effects, in terms of water content, gaseous exchange, and dry weight, of transferring from the grain the embryo of barley to water or to a culture solution are described.

2. The rate of water uptake of the embryo in the seed is lower than that of the isolated embryo when both seed and embryo are floated on a free water surface. The difference is attributed to a low level of water availability in the seed, this being determined by the low permeability of the seed coat for water.

3. The rates of oxygen uptake and of carbon dioxide emission by the embryo of the intact seed floated on water are low. When the embryo is

excised and transferred to water both rates increase. The low rates in the seed are attributed to low levels of water and oxygen availability, determined primarily by the low permeability of the seed-coat membranes to both oxygen and water.

4. The water content and gaseous exchange determinations provide the data for an evaluation of the concentration of oxygen and carbon dioxide with which the contents of the seed are in equilibrium. The estimated average values are 10 per cent. for oxygen and about the same for carbon dioxide.

5. When the excised embryo is transferred either to water or a liquid culture medium there is an immediate drop in dry weight. This, it is suggested, indicates a leaching effect of the fluid. After the initial fall the dry weight continues to decrease, but less precipitately than in the first phase. No comparable decrease in dry weight is observed in the embryo of the intact seed. The difference, it is suggested, is due partly to the higher respiration rate sustained by the isolated embryo.

6. The effect on isolated embryos of the nutrient in the artificial medium is to increase slightly the rates of the exchange, and to promote a slightly higher water content some hours after excision.

7. The effect, in terms of the relations studied, of the time of excision on the subsequent course of development during the experimental period of 12 hours is only slight. After excision the changes follow the same course whatever the time of excision. But since excision induces a more rapid uptake of water and increases both rates of the gaseous exchange it also has the effect of promoting a more rapid development, at least immediately after excision. Thus within the experimental series, at any appropriate time, the embryos excised at any early stage reach a more advanced stage of development than others excised later.

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The Physiology of Incompatibility in Plants

II.   Linum grandiflorum

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With Plate II and three Figures in the Text

INTRODUCTION

SELF-INCOMPATIBILITY in homomorphic plants is commonly controlled by a series of multiple allelomorphs. There are a large number of such allelomorphs in some plants, each of which causes a reaction between the pollen-tube and a style carrying the same allelomorph. These reactions are highly specific, and for this reason they have been compared with the immunity reactions in animals, which depend upon the precipitation of specific proteins or polysaccharides (East, 1929; Sears, 1937). In view of the great number and specificity of these reactions this appears to be the best explanation. Self-incompatibility in distylic plants differs from that in homostyled plants with the S type of inheritance in that there are only two incompatible reactions between pollen and style. Thus two allelomorphs only are concerned in its inheritance, and for that reason it is not necessary for the physiological mechanism causing incompatibility to have great variation of reaction as in the S type. Therefore I expect that, while mechanisms of the S type will always be due to an immunity reaction, in mechanisms of the distylic type other reactions will be possible. Since only two varieties of the incompatibility reaction are used by the distylic system, simple differences of pH or osmotic pressure between the styles and pollen would be adequate for the mechanism.

Linum grandiflorum is distylic, but unlike most distylic plants the level of the anthers is not greatly different in the two types. Although the long- and short-styled flowers are not morphologically identical with pin and thrum flowers in Primula species, they will be called pin and thrum for convenience. Darwin (1877) found that illegitimate matings, i.e. pin×pin and thrum×thrum, were relatively incompatible. Furthermore, he observed that pin pollen did not germinate on pin styles but that thrum pollen readily germinated on pin styles. The present study is an attempt to determine the type of mechanism causing incompatibility in this plant.

POLLEN-TUBE GROWTH IN THE STYLE

The styles of *Linum grandiflorum* are thin, and consequently pollen-tubes can easily be seen by gently squashing the style on a slide and staining in a

mixture of light green and acid fuchsin. Pollinations were made on flowers kept in thermostatically controlled incubators. The stigma is a long papillose surface running up one side of the style, so that to ensure accuracy of pollen-tube measurement the pollen was applied to a small part at the top of the
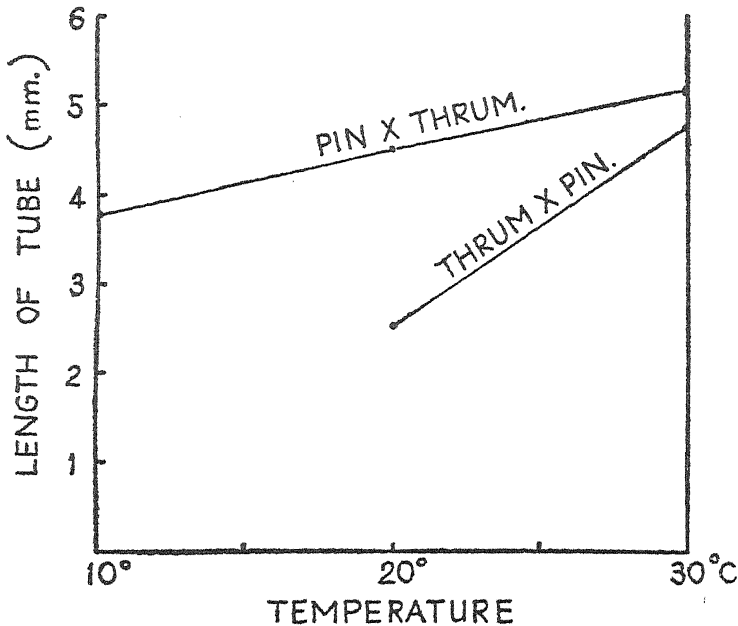
TEXT-FIG. 1.   Length (mm.) of pollen-tubes in legitimate matings, 4 hours after pollination at different temperatures.

stigma. Measurements were then made from the centre of the pollination area on the stigma to the tip of the pollen-tubes. The results of legitimate pollinations, which are given in Text-fig. 1, show that the pollen-tube growth is more rapid at 30° than at 20° C.; this increased growth at higher temperatures is typical of compatible pollen-tubes.

Illegitimate pollinations were studied in selfs and crosses between plants of the same type, the styles were examined for pollen-tube growth at 4 and 24 hours after pollination. In all long-style (pin) pollinations the pollen failed to germinate under any conditions (Pl. II, Fig. 1); in fact, examination of the pollinated styles under the binocular microscope and also in paraffin showed that the pollen was not swollen. In short-style (thrum) pollinations, the pollen-grains germinated and 4 hours after pollination the tubes were 3 times the diameter of the pollen-grain but the tips had swollen and burst (Pl. II, Fig. 2). Temperature has no effect on the growth or time of bursting of these tubes. Thus Darwin's observations are confirmed and explained.

In all other self-incompatible species which have been examined, temperature has a profound effect on the growth of incompatible tubes (Lewis, 1942). At low temperatures (10–15° C.) their growth is relatively fast and is approxi-

mately that of compatible tubes at the same temperature. However, the growth rate of incompatible tubes diminishes as the temperature rises. This effect of temperature is probably due to the increased rate of the immunity reaction between the incompatible pollen-tubes and the style. That *Linum grandiflorum* does not behave in the same way in respect of temperature suggests that the mechanism of incompatibility is different. The bursting of the thrum pollen and the non-swelling of the pin pollen suggests that there is an osmotic difference between the two types of styles.

A point worth noting, although not of immediate significance, is that in Linum the thrum pollen grows nearly twice as fast as the pin pollen in legitimate matings; this difference is also present in *Primula obconica* and *Primula sinensis* (Lewis, 1942). But in the two Primula species the more rapid rate of growth is associated with greater size of the thrum pollen-grain; in *Linum grandiflorum* there is no size difference.

OSMOTIC PRESSURE OF STYLES AND POLLEN-GRAINS

The osmotic pressure within plant cells cannot be measured with great accuracy, but it can be measured accurately enough, in suitable plant material, to show large differences.

The two methods of estimating the osmotic pressure which were used depend upon the cell being impermeable to the osmotically active substance in the solution. The stigmatic cells of the styles were evidently impermeable to sucrose, since plasmolysis, when it occurred, was rapid and recovery by entry of sugar into the cell did not occur. In the *plasmolytic method* the styles are placed in sugar solutions of different concentrations and the solution in which incipient plasmolysis occurs is isotonic with the cell sap. The accuracy of this method depends upon the ease with which incipient plasmolysis can be seen. The stigmatic cells are ideal in this respect since the cell-wall is thin and the sap contains anthocyanin which makes the outline of the protoplast very clear. In the *tissue tension method* styles are placed in different concentrations of sugar solution; in a solution which is isotonic with the cell sap the style does not curl. This method depends upon the two sides of a piece of tissue differing in their permeability to water, so that the tissue curls in one direction if the solution is hypotonic and in the other direction if the solution is hypertonic. The differential permeability on the two sides of a tissue is usually obtained by cutting a pithy stem longitudinally; the epidermis being impermeable and the parenchyma of the pith permeable to water. In the case of the Linum style the structure of the stigma makes such cutting unnecessary. The top 1–2 mm. of the style has a stigmatic surface on one side only (Pl. II, Figs. 3 and 4). This surface is permeable to water, while the epidermal cells of the opposite side are relatively impermeable: when the style is placed in water the top immediately curls up with the stigmatic surface on the convex side. In 30–40 per cent. sucrose there is an equal curling in the opposite direction. In an isotonic solution it remains straight.

The results of the plasmolytic method are given in Table I. Several

different plants of pin and thrum were tested by both methods, and differences between plants within a group were so small that they could not be detected by the methods available.

<div align="center">

TABLE I

Response of Stigmatic Cells to Sucrose Solutions

</div>

| | Degree of plasmolysis. | |
Sucrose (%).	Pin style.	Thrum style.
0	—	—
5	—	—
10	—	—
12	—	+
15	—	+ +
18	+	+ + +
20	+	+ + +
25	+ +	+ + +
30	+ + +	+ + +
50	+ + +	+ + +

The stigmatic cells of the pin style are isotonic with an 18 per cent. sucrose solution with an osmotic pressure of approximately 13·5 atms., while the corresponding values for the thrum style are 12 per cent. and approximately 9 atms.

The results of the tissue tension method are given in Text-fig. 2. The stigmatic cells of the pin style are isotonic with 20 per cent. sucrose, while those of the thrum style are isotonic with 10–12 per cent. sucrose. Since the two methods agree fairly well, there is little doubt that the osmotic pressure of the pin style is considerably higher than that of the thrum style.

Measurement of the osmotic pressure of the pollen-grains was more difficult and consequently less reliable than that of the styles. It was almost impossible to observe plasmolysis because of the thickness of the wall and the absence of pigment in the sap. Furthermore, observations on the pollen-tubes were difficult because the pollen failed to germinate in the usual artificial media. However, an estimate of osmotic pressure was given by the bursting of the pollen-grains. In all solutions of sucrose weaker than 50 per cent. the grains burst immediately. Because of the high concentrations of sucrose required, solutions of potassium nitrate were also used.

Pin pollen is isotonic with 3 per cent. KNO_3 or 50 per cent. sucrose, while the corresponding values for thrum pollen are 5 per cent. KNO_3 and 80 per cent. sucrose (Table II). Similar results were obtained with the pollen-tubes by germinating the pollen on the style and, after 1 hour, placing the style in sucrose solutions. At this time the tubes were in length 2–3 times the diameter of the grain and had barely entered the style. The results confirm those previously described and give additional evidence in the following way. Thrum pollen, after it has germinated on its own style, bursts more quickly in sugar solution than after it has germinated on a pin style. This is presumably due to the effect on the pollen-grain of the low osmotic pressure of the thrum style.

Thus, the pin plant has styles with a high osmotic pressure and pollen with a low osmotic pressure, while the thrum plant has a low osmotic pressure in the styles and high in the pollen grains. Furthermore, in both forms the pollen has a very much higher osmotic pressure than the styles. This latter point has been observed in other plants, by Tischler (1917), Renner (1919), and Walderdorff (1924).
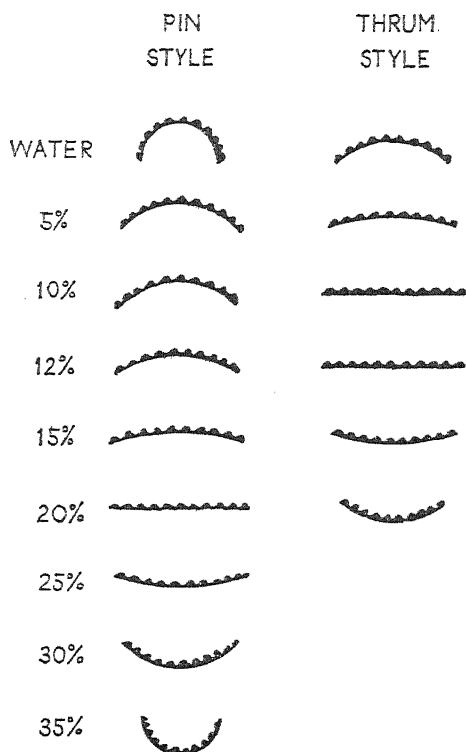
TEXT-FIG. 2. Curvatures of pin and thrum styles in different concentrations of sucrose. The stigmatic surface is represented by a wavy line.

TABLE II. *The Effect of different Concentrations of Sucrose and KNO_3 on the Bursting of Pollen*

	KNO_3			Sucrose	
	pin	thrum		pin	thrum
%	% bursting.		%	% bursting.	
2	100	100	5	100	100
			10	,,	,,
			15	,,	,,
3	7	44	20	,,	,,
			25	,,	,,
4	7	18	30	,,	,,
			50	5	62
			65	0	40
5	0	0	80	plasmolysed	5

STYLE MOVEMENT AFTER POLLINATION

Darwin (1877) found that the pin styles, when pollinated with thrum pollen, became twisted, while with pin pollen they remained straight. The twisting of the pin style when legitimately pollinated is rapid and a strong curvature is present after 15 minutes (Pl. II, Fig. 6). This different behaviour of the pin styles to the two types of pollen can now be explained. The pin pollen does not germinate or swell on the style and therefore it does not absorb water from the stigmatic cells. Consequently the style does not curl. The thrum pollen germinates and absorbs water from the stigmatic cells, hence the curling of the style. The thrum style curls after illegitimate pollination (Pl. II, Fig. 5). The pin style after illegitimate pollination remains straight. This difference is to be expected from the behaviour of the two types of pollen. The curling of the style is due to a withdrawal of water from the stigmatic cells; this is shown by placing in a 5 per cent. sucrose solution a style which has been curled previously by pollination. The style then straightens and curls in the opposite direction, with the stigmatic cells on the convex side.

The pollen-tube observations, the osmotic pressure measurements, and the curling of the style all show that the incompatibility which accompanies illegitimate pollinations is due to a disparity in the osmotic pressure between styles and pollen. The results are summarized in Text-fig. 3 to show this agreement.

However, the osmotic pressure difference is not the full explanation. Since the osmotic pressure of the pin pollen is more than twice that of the style, it is remarkable that the pollen is unable to withdraw water from the stigmatic cells of its own style. Can this be attributed to the turgor pressure of the pollen-grain wall? If we consider the legitimate matings we find that the ratio of the osmotic pressures of pollen and style is 4 : 1 in both pin × thrum and thrum × pin matings. In illegitimate matings, in which the ratio is 5 : 2 in the pin × pin, pollen does not absorb water; but when it is 7 : 1 in thrum × thrum the tubes burst. This suggests that the water relations of the pollen and style are delicately balanced, perhaps by the turgor pressure of the wall; the difficulty in germinating Linum pollen in artificial media supports this. Nevertheless, turgor pressure of the wall alone does not explain the inability of the pollen to extract water from the style. For, as we have seen, water is withdrawn from the pin style by a 25 per cent. sucrose solution and pin pollen can withdraw water from the same concentration of sucrose. Therefore, pin pollen should, on simple osmotic pressure relations, withdraw water from the style.

Dr. K. Mather suggested to me that a rescaling of the osmotic pressures might obviate this apparent inconsistency. If the osmotic pressures of the pollen-grains are divided by 4, we get a ratio of 2 : 1 for pollen : style in thrum × thrum and 1 : 2 in pin × pin. Thus the non-swelling of pin pollen and the bursting of thrum pollen are both consistent with the rescaled ratios. This division by 4 has some meaning because for normal pollen-tube growth in Linum the osmotic pressure of the pollen-grain is 4 times that of the style.

The explanation for this difference may lie in the nature of the protoplasmic colloids. The uptake of water by plant cells is not only a process of simple osmosis but imbibition by protoplasmic colloids (cf. Stiles, 1924). Imbibition is probably more important than osmosis in the swelling of the pollen-grain

PXT	TXP	PXP	TXT

O. P. RATIO   4 : 1   5 : 2   7 : 1
POLLEN: STYLE.
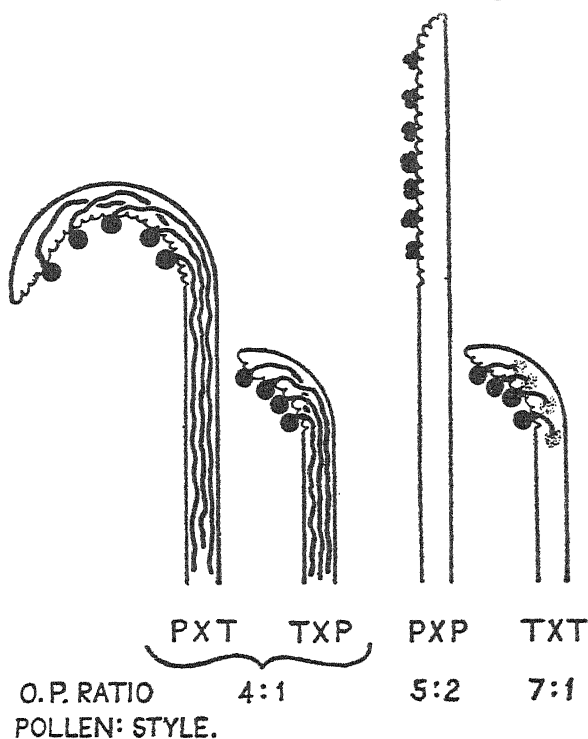
TEXT-FIG. 3. Diagram showing the relation between the osmotic pressure and the behaviour of the pollen and styles.

and early growth of the tube, but osmosis plays a more important part in later growth (Brink, 1924). The incompatibility system in *Linum grandiflorum* therefore depends upon osmotic pressure differences and probably differences in the nature of the cell colloids.

SUMMARY

1. Incompatibility in illegitimate matings in *Linum grandiflorum* is due to the pollen-grains failing to swell and germinate in pin × pin pollinations, and to the pollen-tubes bursting in thrum × thrum pollinations. Low temperature, which in other plants causes a higher rate of growth of incompatible tubes, has no effect in Linum.

2. Compatible pollen-tube growth is normal, the rate increasing with higher temperatures. Thrum pollen grows approximately twice as fast as pin pollen, although there is no size difference.

3. The twisting of the pin style following legitimate pollination, and of the thrum style after all pollinations, is due to the withdrawal of water from the style by the pollen. Pin styles when illegitimately pollinated do not curl because the pollen does not extract water from the stigma.

4. The osmotic pressure of the styles, as measured by the plasmolysis and tissue tension methods, is equivalent to 10–12 per cent. sucrose in the thrum styles and to 20 per cent. sucrose in the pin styles. The values for the pollen-grains are—thrum pollen 80 per cent. sucrose, pin pollen 50 per cent. sucrose.

5. The ratio of the osmotic pressure of pollen to styles is 4 : 1 in both legitimate pollinations, 5 : 2 in pin × pin and 7 : 1 in thrum × thrum. This disparity in osmotic pressures partly determines the behaviour of illegitimate pollen-tubes.

6. The inability of the pin pollen to extract water from the stigma cannot be explained entirely on unsuitable osmotic pressure differences, and it is suggested that differences in the protoplasmic colloids may control the imbibition of water in the early stages of pollen germination.

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PLATE II

Illustrating the article by Dr. D. Lewis on 'The Physiology of Incompatibility in Plants. II. *Linum grandiflorum*'

Fig. 1. Three styles from the same flower of a pin plant; the lowest one was pollinated with thrum pollen. The other two were self-pollinated; the pollen did not germinate and was washed off. (× 109.)

Fig. 2. Three styles from the same flower of a thrum plant; the uppermost one was pollinated with pin pollen, the other two were self-pollinated. (× 109.)

Fig. 3. Pin style in 20 per cent. sucrose solution. The stigmatic cells are plasmolysed and are on the concave edge. (× 46.)

Fig. 4. Pin style in 5 per cent. sucrose solution. The stigmatic cells are turgid and are on the convex side. (× 46.)

Fig. 5. A thrum style which has been self-pollinated, showing the curvature of the style after 15 minutes. (× 46.)

Fig. 6. Pin style crossed with thrum pollen, showing curvature of the style after 15 minutes. (× 46.)

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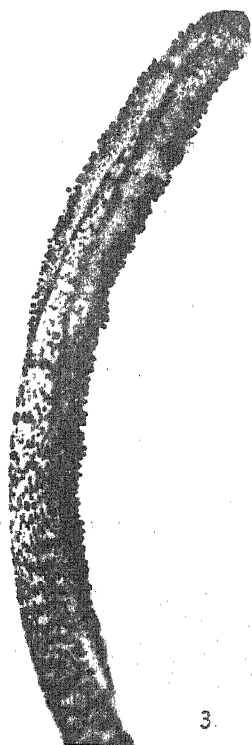
3.

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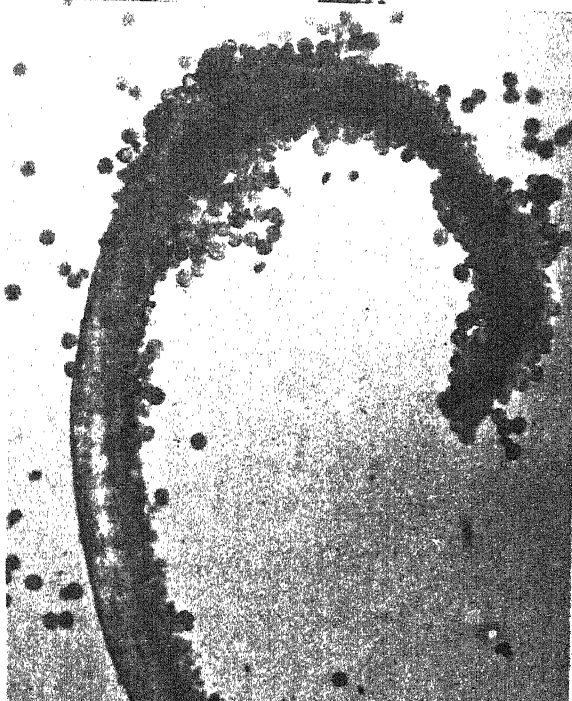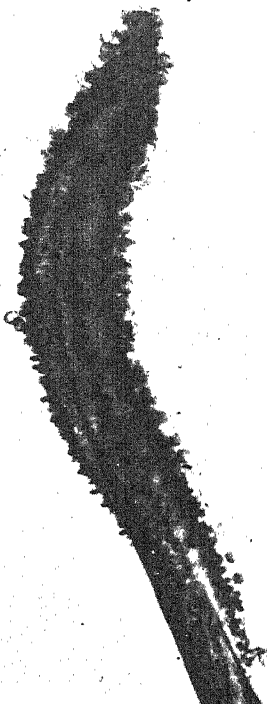
The Effect of Nutrition and Phytohormones on the Rooting of Vine Cuttings

BY

HAROLD L. PEARSE

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With Plate III

INTRODUCTION

IT is now well known that by treatment with plant hormones root formation in the cuttings of many species of plants is accelerated and the number of roots produced increased. Recent researches by a number of workers in all parts of the world have shown, however, that a number of species fail to respond to such treatment. In general it has been found that species which root fairly readily without treatment respond almost without exception to the hormone by an increase in the number of roots formed and a decrease in the time taken to form roots. With species, however, which are very difficult to root from cuttings only a very small percentage show any response to hormone treatment; a high percentage of our most valuable horticultural species both ornamental and domestic belong to this class. It is not surprising, therefore, in view of the practical importance of the problem that numerous attempts have been made by altering such factors as the constitution of the rooting medium, the method of application and concentration of the hormone, and the varying of other external factors during the rooting period, to improve the rooting of these difficult species. These efforts have in the majority of cases been attended with but slight success, and it is therefore probable that with these species some other factor as yet unknown is limiting root formation. In a few cases the addition of carbohydrates and of vitamin B_1 has been found to effect an improvement; but such treatments also are only of limited application to isolated cases. Many investigators (Biale and Halma, 1938; Gocolašvili and Maximov, 1937; Hubert, Rappaport, and Beke, 1939; Komissarov, 1938; Lek and Krythe, 1937; Longley, 1939; and Scholz, 1937) have observed, however, that the physiological condition of the material used for cuttings is a very important factor in governing their response to applied hormones. Pearse (1938) using softwood deciduous fruit-tree cuttings found with species of apple, pear, plum, and cherry that cuttings gathered in the late summer from shoots in which the terminal bud had stopped growth rooted and responded to hormone applications much more readily than cuttings taken earlier while the shoots were still in active growth, though this was not invariably the case with all

species investigated. The response to hormone treatment is thus dependent on the appropriate condition of the cuttings, which seems to point to some other factor controlling root formation after treatment. It is common knowledge among nurserymen that in one locality a given species will root readily while in another locality great difficulty is experienced. It is probable, therefore, that the composition of the parent plant is a most important factor in controlling the root formation of cuttings derived from it.

Reid (1924 and 1924 *a*), Starring (1923), and Schrader (1924) found that a high carbohydrate content of tomato cuttings plus a relatively low supply of soluble nitrogen favours the regeneration of roots. Further, the time at which the terminal bud is formed on the shoot of deciduous fruit-trees is the time at which a state of internal starvation is beginning to be evident in the tree; the rate of absorption of nutrients by the roots can no longer keep pace with the shoot growth and therefore there is an accumulation of carbohydrates and a high carbohydrate/nitrogen ratio in the shoots.

The question therefore naturally arose as to whether it is possible by adjusting the composition of the parent plant by cultural treatments to improve the rooting and response to phytohormones of cuttings derived from it. The present work, which is in the nature of a preliminary investigation, is concerned with the effect of the mineral nutrition of vine plants on the ability of cuttings from them to produce roots, both with and without applied hormones, and also of the effect of the addition of soluble nitrogen to the rooting medium.

MATERIAL AND METHODS

One-year-old plants of *Vitis vinifera* (var. Waltham Cross) were set in pure quartz sand contained in 5-gallon glazed earthenware crocks, standing in the open; these were cut back to two buds at planting. There were four series, each consisting of four plants, which were fed respectively with the nutrient solutions shown in Table I.

TABLE I

Composition of the Nutrient Solution

Series.	Salts Total (p.p.m.).	Molar concentration.			
		KNO_3	$Ca(NO_3)_2$	KH_2PO_4	$MgSO_4$
A	171·2	0·0005	0·0005	0·0000525	0·00025
B	684·8	0·002	0·002	0·00025	0·001
C	2739·2	0·008	0·008	0·001	0·004
D	10956·8	0·032	0·032	0·32	0·016

In addition to the salts shown in the table a solution containing boron, manganese, zinc, and copper at 0·5, 0·5, 0·05, and 0·02 p.p.m. respectively was added, and iron was supplied as ferric tartrate (1 c.c. of a 0·5 per cent. solution per litre of nutrient solution). One litre of solution was supplied to each pot three times a week throughout the growing season.

The growth of the vines.

The vines grew well under these conditions, and the only visual symptoms of deficiency in any of the plants were in series A receiving the most dilute solution; here symptoms of nitrogen deficiency were clearly evident, the leaves being small and light green in colour, and the growth stunted. The vines growing in the most concentrated solution (series D) made a second flush of growth in the autumn when some of the buds formed during the current season began to grow out, especially at the base of the vine, producing a second crop of leaves and flowers. The average shoot growth produced per single vine in each series was as follows: A, 180 cm.; B, 302 cm.; C, 531 cm.; and D, 424 cm. The internodes were much shorter in series A, and the buds were much smaller in size.

Preparation of the material for rooting tests.

In the winter of the following year the vines were removed, and the dormant shoots so obtained were divided into cuttings consisting of a single bud and the greater part of the internode below it. The cuttings were then set in pure quartz sand in open tins in a glasshouse. Half of the cuttings were planted without any treatment, and the other half had their basal portions dipped in a talc powder containing 2 mg. per gm. indolylbutyric acid; the powder was prepared by the method of Stoutemyer (1939). The cuttings were watered freely when necessary, and the water ran readily through the sand, thus giving a continual and plentiful supply of air to the rooting medium without water-logging. Later, when the buds were just beginning to push, half of the control and half of the hormone-treated cuttings were watered with a solution of KNO_3 containing 200 mg. of the salt per litre.

Results.

The cuttings were set in the sand on August 15, and by September 1 many of the buds had broken their dormancy and were beginning to grow out. At this date all the buds from series D (the most concentrated solution) were still dormant. On September 19 a count of buds growing and buds still dormant was taken, and the results are shown in Table II.

TABLE II

Growth of the Buds on September 19

	Control.		Indolylbutyric acid.	
Series.	Number of buds growing.	Buds dormant (%).	Number of buds growing.	Buds dormant (%).
A	40	13	38	17
B	41	18	47	6
C	44	26	41	31
D	19	52	13	67

It is clear from the above table that the dormancy of the buds was prolonged by increasing the concentration of the nutrient solution (series A to D). The

hormone treatment still further inhibited the 'breaking' of the buds in all series except B. It was also observed that the buds remaining on the parent plants exhibited the same behaviour in breaking as those on cuttings.

ROOT AND SHOOT FORMATION

On September 19 half of the cuttings in each series were watered with a solution of KNO_3 (200 mg. per litre) and this treatment was continued weekly until October 13, when the cuttings were removed from the sand and carefully washed for examination. The number of roots on each cutting was counted and their length measured; the results are shown in Table III.

TABLE III

Growth of the Cuttings

Treatment.	Series.	No. cuttings.	% rooted.	% buds dead.	No. roots per cutting.	Length of roots per cutting (cm.)
Control	A	23	100	9	3·8	8·0
	B	25	96	20	4·1	6·9
	C	30	70	23	2·7	2·4
	D	20	70	25	2·7	3·5
KNO_3	A	23	96	9	3·6	7·0
	B	25	100	0	3·8	6·1
	C	30	73	13	2·4	3·1
	D	20	74	17	4·5	6·4
Indolylbutyric Acid	A	23	96	13	5·0	13·4
	B	25	100	0	7·0	17·1
	C	30	70	17	3·5	4·9
	D	20	70	35	3·6	4·5
Indolylbutyric Acid plus KNO_3	A	23	100	0	7·8	25·6
	B	25	100	0	9·9	32·3
	C	30	61	30	2·7	3·2
	D	20	60	60	5·3	7·7

"Rooted cuttings only." spans the last two columns (No. roots per cutting and Length of roots per cutting (cm.)).

Untreated cuttings.

The leaves produced by the cuttings derived from the parent plants which were grown in the dilute solution A were small, light green in colour, and showing signs of protein breakdown.

Progressing from series A to D the cuttings bore larger and greener leaves; in series D the leaves were exceptionally large and well coloured. There was, however, an increasing percentage of bud mortality from series A to D, although a number of the cuttings in which the bud had died produced a few roots. A higher percentage of the cuttings rooted from series A and B, as compared with those derived from parent plants in series C and D which received the concentrated nutrient solutions, and, moreover, there was a greater number of roots per cutting and the roots were very much longer (Pl. III, Figs. 1 and 2).

Cuttings receiving KNO₃ alone.

The addition of KNO_3 to the rooting medium slightly improved the colour of the leaves in series A and B, but had very little effect on either the number of cuttings rooting, the number of roots per cutting, or the length of the roots produced; it was somewhat detrimental in all series except D, where a slight improvement was observed. The amount of bud mortality was, however, diminished (Pl. III, Figs. 3 and 4).

Cuttings receiving indolylbutyric acid.

The hormone had little effect on leaf colour, bud mortality was increased in series A and D and decreased in series B and C. The hormone was effective in causing the production of more roots per cutting, and the roots were much longer, especially in cuttings derived from series A and B (Pl. III, Figs. 5 and 6).

Cuttings receiving indolylbutyric acid plus KNO₃.

The colour and the size of the leaves of the cuttings from series A and B were much improved, and bud mortality was completely prevented; in cuttings from series C and D, however, bud mortality was greatly increased. Furthermore, in cuttings from the A and B series all the cuttings rooted and the number and length of the roots were very greatly increased, whereas in cuttings from series C and D the treatment caused a drop in the percentage of cuttings rooting and had little effect on either the number or the length of the roots formed (Pl. III, Figs. 7, 8, and 9).

TESTS WITH FIELD MATERIAL

Vines of three rootstocks, namely 420 A, 333, and Jaquez, were obtained from the experimental farm at Groot Drakenstein. These rootstocks are reported by the Viticulture Department of this research station as being rather difficult to root from cuttings, and giving a poor response to applied hormone (indolylacetic and indolylbutyric acids), the rooting and 'take' usually obtained under field conditions varying between 20 and 40 per cent. Single bud cuttings were prepared from this material and their further treatment was similar to that already described. Of each variety there were four series of forty cuttings receiving ·the following treatments respectively, (1) control, untreated; (2) KNO_3 (200 mg. per litre) applied weekly to the sand, after the buds had started to grow out; (3) indolylbutyric acid (2 mg./g. in talc dust) applied at planting; and (4) treatments 2 and 3 combined. The cuttings were set in the sand on September 10, and on November 12, after 9 weeks in the rooting medium, they were removed and the number and length of the roots determined. The results are shown in Table IV.

All the cuttings rooted except in the case of Jaquez, in which a small number of the untreated and nitrate-treated failed to root. The treatment with indolylbutyric acid in conjunction with KNO_3 added to the sand was consistently the

TABLE IV

The Reaction of Cuttings of Vine Rootstocks to Treatment with Indolylbutyric Acid and KNO_3 (40 cuttings in each treatment)

Variety.	Treatment.	Number rooted (%).	Roots per cutting.	Length of roots (cm.).
420 A	Control	100	4·5	7·6
	KNO_3	100	3·3	7·1
	Hormone	100	4·8	13·4
	Hormone + KNO_3	100	4·3	16·8
333	Control	100	5·9	12·6
	KNO_3	100	7·6	20·0
	Hormone	100	8·2	23·3
	Hormone + KNO_3	100	10·6	33·2
Jaquez	Control	80	1·8	6·6
	KNO_3	80	1·5	4·6
	Hormone	100	2·4	7·4
	Hormone + KNO_3	100	3·3	14·5

best, the length of root per cutting being greatly increased; with the exception of 420 A the cuttings produced more roots, and this was also true to a lesser degree with the treatment with indolylbutyric acid alone. The treatment with KNO_3 alone was detrimental with varieties 420 A and Jaquez, and slightly beneficial with 333.

DISCUSSION

The present study has clearly shown the importance of the nutrition of the parent plant in governing the capacity of cuttings derived from it to produce roots. Thus vine plants growing under starvation conditions, especially for nitrogen, gave cuttings which rooted readily while producing small shoot growth. With adequate or excessive nutrition of the parent plant the cuttings so obtained produced few roots even though there was abundant leaf growth; there was, moreover, a relatively high rate of bud mortality in the cutting bed. These results are in agreement with those of Reid (1924 and 1924 *a*), Schrader (1924), and Starring (1923) who worked with softwood tomato cuttings severed from the parent during the growing period; they support the view that a high carbohydrate/nitrogen ratio in the cutting material is favourable for the production of roots. Thus in cuttings derived from series B (moderate nitrogen starvation) supplying soluble nitrogen in small amounts to the rooting medium tended to decrease both the number and the length of the roots produced.

The response of the different types of material to the applied hormone was very striking; thus the cuttings derived from the starved plants responded exceptionally well to hormone treatment, both the number and the length of the roots being greatly increased, whereas those derived from plants which had been growing at a moderate or high level of mineral nutrition showed a very slight response. It is evident, therefore, that in the latter types of cutting

the lack of root-forming substance is not the major factor limiting root-formation, and that some other factor at present obscure is responsible for the poor root production. It seems improbable that this factor is carbohydrate supply, since these cuttings produced abundant and large leaves which should have ensured a continual and adequate supply of carbohydrate to the cuttings. On the other hand, the starved material, which formed roots readily without hormone treatment, responded well to this treatment, the number and length of the roots being greatly increased by the addition of the indolylbutyric acid. In this case, therefore, the amount of root formation was limited by the availability of the root-forming substances. It is interesting to note that this is precisely similar to the response to hormone treatment of easily rooting and difficult-rooting species. With species which are easy or only moderately difficult to root without treatment, optimal hormone treatment rarely fails to result in increased and more rapid root formation; with species normally very difficult to root, however, a hormone treatment in the majority of cases fails to increase appreciably the number of individuals rooting. With these difficult species, however, a response to hormone treatment is sometimes obtained when cuttings are taken from particular individuals, or the mother plant is in a particular stage of growth. Thus, for example, with many varieties of fruit-trees Pearse (1938) has shown that with softwood cuttings the optimum response to hormone treatment is obtained in the late summer when the shoots have stopped growth, a stage at which a condition of internal mineral starvation, especially of nitrogen, is preventing further growth in length. This variation in response to root-forming substances may well be due to fluctuations in the level of the unknown factor postulated earlier.

Doak (1940) found a number of nitrogenous compounds, mostly amino-acids and purins, to promote rooting in cuttings of a species of Rhododendron, while Grace (1939 and 1940) noted an improvement in the rooting of spruce cuttings caused by giving a full nutrient solution in addition to a hormone treatment; and Thimann and Poutasse (1941) observed that rooting of leaf cuttings of *Phaseolus vulgaris* is greatly promoted by the addition of KNO_3, whereas $(NH_4)_2SO_4$, even at high dilution, almost entirely prevented root formation. In the present study it was found that with cuttings obtained from nitrogen-starved plants a supply of soluble nitrogen added as KNO_3 to the rooting medium had little or even a slightly detrimental effect on root formation if applied alone; but applied in conjunction with indolylbutyric acid it was effective in still further increasing both the number and the length of the roots formed. In contrast with material obtained from plants receiving an abundant or excessive mineral supply, a treatment with KNO_3 alone had little effect, and supplied in conjunction with the hormone it decreased the number of cuttings which rooted and increased the number of buds dying in the rooting medium. It is clear, therefore, that with nitrogen-starved material in the presence of abundant root-forming hormones the lack of soluble nitrogen can act as a limiting factor for root formation; but the absence of additional nitrogen is not in itself a limiting factor, as adding KNO_3 alone had if anything

a detrimental effect. With material from plants receiving an abundant mineral supply the addition of KNO_3 and indolylbutyric acid, either alone or in conjunction, had little effect on root formation which was generally very poor in such material; and the cause of the failure of such material to form roots is not yet fully elucidated.

From the practical viewpoint these results seem to indicate that material cultivated especially for propagation by cuttings needs special nutritional treatment, and should presumably be grown on a poor soil low in nitrogen; cuttings obtained from such material should then be treated with root-forming substances and transferred to beds containing a readily available supply of soluble nitrogen. The vine is of course relatively easy to propagate, and it is impossible to say as yet whether these findings will apply in general to difficult-rooting species; but a promising further line of investigation has been opened up and the importance of the composition of the parent plant clearly shown. It is undoubted that plants growing on a poor soil, low in available mineral nutrients, have the power of producing an abundant root system; also the ratio of the amount of root to the amount of shoot growth is much higher than that in plants growing on a rich soil, and thus the plant is enabled to explore a much larger area of soil. This fact has recently been shown clearly by Eaton (1941), who grew maize and tomato plants with their roots divided between two or more solutions of unequal concentration. They developed more roots in the dilute than in the concentrated solutions, and this was found to be the case irrespective of whether differences in concentration were affected by the addition to the base nutrient of chloride, sulphate, or additional nutrient salts. Eaton considered, therefore, that osmotic pressures, rather than specific ion effects, are primarily involved, and this is undoubtedly a factor which must be considered in relation to the present study, for from series A to D the mother plants were growing in solutions of increasing osmotic pressure, and further work will therefore be necessary to clarify this point. It is clear, however, that when plants are growing under conditions which induce the formation of an abundant root system, this property is retained by the cuttings severed from them which exhibit it by regenerating a great number of quickly growing roots.

With the field material of three varieties of vine rootstocks the beneficial effect on the root system of the cuttings of a combined treatment with KNO_3 and indolylbutyric acid was clearly demonstrated. With a hormone treatment alone an appreciable response was obtained, but except in the case of one variety the number of roots produced was not greatly increased. It seems probable, therefore, that this material was intermediate in composition between that obtained from the sand cultures B and C. The propagation by single bud cuttings proved to be a useful method of rapidly increasing stocks of valuable material.

SUMMARY

Plants of *Vitis vinifera* (var. Waltham Cross) were grown for a single season in a series of sand cultures, the different series being fed with nutrient solu-

tions of increasing concentration. In the following winter single bud cuttings were taken from the dormant vines and the regeneration of roots in these cuttings was studied.

1. Cuttings from plants grown under conditions of mineral starvation or semi-starvation, and exhibiting symptoms of nitrogen starvation, (a) bore small pale green leaves, and rooted readily; (b) responded to the addition of a solution of KNO_3 to the rooting medium by an improvement in leaf size and colour and a diminution in the amount of root formation; (c) responded well to treatment with indolylbutyric acid, both number and length of the roots being increased; (d) responded exceptionally well to a treatment with indolylbutyric acid *plus* the addition of KNO_3 to the rooting medium, leaf size and colour being improved and the number and length of the roots being very greatly increased.

2. Cuttings from plants grown under conditions of adequate or excessive nutrition, and making very vigorous growth (a) bore large green leaves, and rooted very poorly; (b) did not respond by improved root formation when KNO_3 was added to the rooting medium; (c) responded poorly to treatment with indolylbutyric acid, neither root number or length being increased to an appreciable degree; (d) did not respond by increased root formation to a treatment with indolylbutyric acid plus the addition of potassium nitrate to the rooting medium, and moreover the mortality of the cuttings was increased by this treatment.

It is concluded that the nutritional conditions and method of culture of the parent plant is of great importance in determining the ability of cuttings to form roots and to respond to treatment with plant hormones. Where hormone treatment is effective a still further improvement is caused by adding KNO_3 to the rooting medium. It is also indicated that when plants are growing under conditions which induce a relatively high proportion of root to shoot growth the tendency to form roots becomes a property of such materials, for portions severed from them have the power of regenerating plentiful and quickly growing roots.

A combined treatment with indolylbutyric acid and KNO_3 greatly improved the rooting of cuttings of three varieties of vine rootstocks obtained from field material.

In conclusion, the author wishes to thank the Director, Dr. M. S. du Toit, for his unfailing interest in this investigation, and the Viticulture Department for providing material of the three rootstocks used.

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EXPLANATION OF PLATE III

Illustrating Dr. Pearse's paper on 'The Effect of Nutrition and Phytohormones on the Rooting of Vine Cuttings'.

Fig. 1. Untreated cuttings from plants grown under conditions of poor nutrition. The upper row from culture series A, the lower from series B.

Fig. 2. Untreated cuttings from plants grown under conditions of adequate or excessive nutrition. The upper row from culture series C, the lower from series D.

Fig. 3. Cuttings received KNO_3 (200 mg. per litre) applied to the rooting medium. Upper row series A, the lower series B.

Fig. 4. Cuttings receiving potassium nitrate 200 mg. per litre applied to the rooting medium. The upper row from series C, the lower from series D.

Fig. 5. Cuttings dipped before planting in talc powder containing 2 mg. per gr. indolyl-butyric acid. Upper row from culture series A, the lower from series B.

Fig. 6. Cuttings dipped before planting in talc powder containing 2 mg. per gr. indolyl-butyric acid. Upper row from series C, the lower from series D.

Fig. 7. Cuttings treated with indolylbutyric acid in talc, and KNO_3 (200 mg. per litre) was added to the rooting medium. Culture series A.

Fig. 8. Cuttings treated with indolylbutyric acid in talc, and KNO_3 (200 mg. per litre) was added to the rooting medium. Culture series B.

Fig. 9. Cuttings treated with indolylbutyric acid in talc, and KNO_3 (200 mg. per litre) was added to the rooting medium. Upper row from culture series C, lower row from series D.
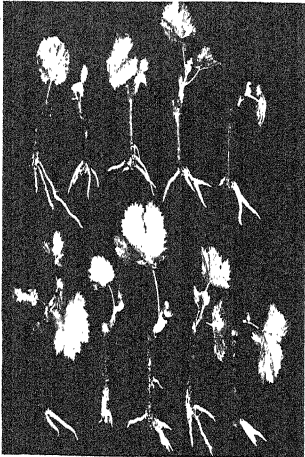
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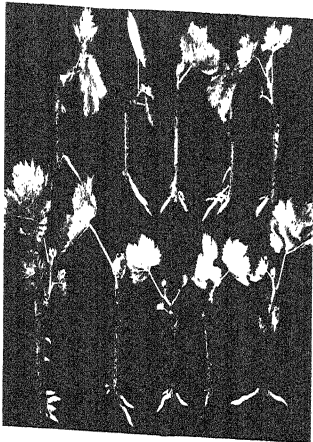
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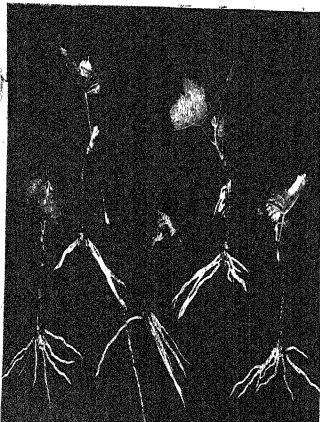
On the Occurrence of Buds on the Leaves of Botryopteris hirsuta Will.

BY

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With Plate IV and five Figures in the Text

I. INTRODUCTION

THE small fossil fern which forms the subject of this paper is one of the commonest and best-known representatives of the Filicales in the Upper Carboniferous flora. It has, therefore, received much attention, along with other members of this interesting genus, by various palaeobotanists from time to time. In spite of this, certain features of its morphology still remain obscure. The object of this paper is to record and describe one such feature which has apparently remained unnoted hitherto. This is the frequent and apparently regular occurrence of buds borne singly near the bases of the pinnae where the latter join the relatively main rachis of a leaf. Numerous buds of this type have been found on the fragmentary remains of leaves present in certain coal-balls. The attachment, however, of the fairly large rachises or petioles to their parent stems has not been seen, nor have specimens been obtained showing the presence of more than one bud on the same fragment of leaf.

Since it is not possible at present to make a full comparative study of this and of other features in the morphology of *B. hirsuta*, it seems advisable to give a short account of the relation of the bud to the leaf bearing it, as shown in a typical example. Hence the present description will be based mainly on a series of transverse sections and reconstruction of a single specimen, referred to as specimen A. Reconstructions of two additional specimens, designated B and C, are added by way of confirmatory evidence of the same relation of parts, and also to give some idea of the variation in degree of development met with in the numerous examples of these buds examined. It is hoped that these examples will suffice to establish the main facts; comparative discussion is purposely left over at this stage.

Williamson (1889, p. 161) described under the name of *Rachiopteris hirsuta* certain specimens of this fern from Oldham. Prior to this, however, Felix (1886) had discovered isolated petioles in Westphalia, which he described under the name of *Rachiopteris tridentata*. Later Scott (1898) assigned *Rachiopteris hirsuta* Will. to Renault's genus Botryopteris, established in 1875 for the reception of the French species *B. forensis*. Hirmer (1927, p. 533) used the

name *B. tridentata* for the plant which Scott (1920, p. 339) described under the name *B. hirsuta* Will. Since the name *B. tridentata* has also been used in a collective sense for isolated petioles of possibly more than one species (cf. Koopmans, 1928, p. 33), it will be the clearer procedure to use the name *B. hirsuta* Will. for the specimens described here. Further records of the occurrence of this species in Holland and Belgium have been made by Koopmans (1928, p. 32) and Leclercq (1925, pp. 58–9). Hoskins (1930) has recorded petioles in the McLeansboro formation of Illinois, though Corsin (1937, p. 189) has identified them with *B. renaulti* Bertrand and Cornaille. Apart from these records and the various text-book accounts, notably those of Seward (1910, p. 438), Scott (1920, pp. 337–42), and Hirmer (1927, p. 533), no more detailed investigation of *B. hirsuta* Will. has apparently been made.

II. Material and Method of Investigation

Coal-balls from the Lower Coal Measures of Lancashire were used as the source of material for studying these buds. The specimens to be described occurred along with others, in a single large coal-ball, obtained from the Union or Mountain Mine at Old Meadows Colliery, Bacup. The first specimen to reveal the true nature of the buds was found in a nodule collected on old tips at Dulesgate, Todmorden. There is every reason to believe from the occurrence of examples in other localities that these buds were of quite general occurrence in the Upper Foot and Union Mines of Lancashire and in the equivalent seam known as the Halifax Hard Bed of Yorkshire.

The specimens were discovered by splitting the coal-balls and etching the exposed surface with HCl. After grinding and polishing, the buds were examined by serial sections made according to Walton's peel method. For the grinding and polishing successively finer grades of carborundum powder were used. The film pulls were made from a solution of celluloid in equal parts of amyl acetate and acetone. The polished surface of the coal-ball was etched by means of 2 per cent. HCl allowed to act for two or three minutes; after washing under the tap and allowing to dry, the etched surface was flooded with amyl acetate and the solution of celluloid carefully poured on from the edge. The time required for drying at room temperature was from 12 to 15 hours. After removal of the film the surface of the coal-ball was either polished again directly, or first slightly ground with carborundum powder (No. 220) and then polished. Before etching and making a fresh film the amount removed by grinding or polishing was determined by measuring the length of the block with a pair of sliding vernier calipers. This was repeated each time a film was made and the difference due to grinding so estimated; only with the help of such measurements could accurate reconstructions to scale be made. The films were placed in HCl to remove adhering mineral matter and then dried and mounted in Canada balsam. Camera-lucida drawings of the principal sections were made, and from them the reconstructions shown on Text-figs. 3, 4, and 5 were built up.

III. Description of Specimen A

(a) The serial sections.

In describing this specimen the structure as revealed in the sections will be first considered, after which it will be possible to describe the position, external form, and size of the bud as deduced from the reconstruction. The series comprises eighty obliquely transverse sections numbered as they were made, from below upwards. These numbers will be used for reference to individual sections. It may be mentioned, however, that all the sections figured on Text-figs. 1 and 2 and Pl. IV are now in the Manchester Museum collection of fossil slides where they comprise a series bearing the numbers R1672 to R1699. The main features of these principal sections are given by the camera-lucida drawings reproduced on Text-figs. 1 and 2, and their chief anatomical details are shown on the photographs of Pl. IV.

In the first section of the series (Text-fig. 1 and Pl. IV, Fig. 1) the main rachis or petiole is cut below the level of the bud. As seen from the section, the adaxial surface is slightly concave and the lateral edges are rounded, in contrast with the condition in *B. ramosa* where usually the mature petiole is approximately D-shaped in cross-section. A narrow epidermal layer of cells is present which is poorly preserved and occasionally bears a few multicellular hairs. Outer and inner zones of the cortex can be distinguished, the outer cortex being a narrow zone of small thick-walled cells. This condition of the cortex is characteristic of *B. hirsuta*, and may be contrasted with that of *B. ramosa*, in which the cortex is typically more uniform with only a slight and gradual decrease in size of the cells towards the outside. In the vascular bundle the phloem is largely decayed, leaving a cavity surrounding the xylem which is in the form of a solid tridentate strand. The three xylem teeth, which project more prominently than in *B. ramosa*, lie towards the adaxial surface, and are composed at their apices, of small tracheides which doubtless represent the protoxylem. The metaxylem is mainly composed of relatively large tracheides measuring 0·22 mm. in maximum diameter. A single lateral lobe of the xylem strand is seen to be separating, apparently as a pinna-trace. When this trace is followed, however, it is seen to divide and supply both the pinna and bud; thus it will be referred to, simply, as the common trace. After dividing, the abaxial portion of this common trace passes to the pinna and the adaxial portion to the bud (see section No. 18, Text-fig. 1). The photographs (e.g. Pl. IV, Fig. 3) show that these two portions of the divided common trace become markedly distinguished by the different size of their component tracheides, those of the bud-stele being evidently smaller. Two pinnules are borne on opposite sides of the pinna. The first, which is on the lower side, has apparently become adnate to the bud, from the base of which it appears to arise as an exogenous branched appendage. Its vascular supply originates from that portion of the common trace which separates as the bud-stele. The second pinnule is borne farther out on the pinna, near the broken or decayed distal end, and on the upper surface. The bud bears numerous roots especially
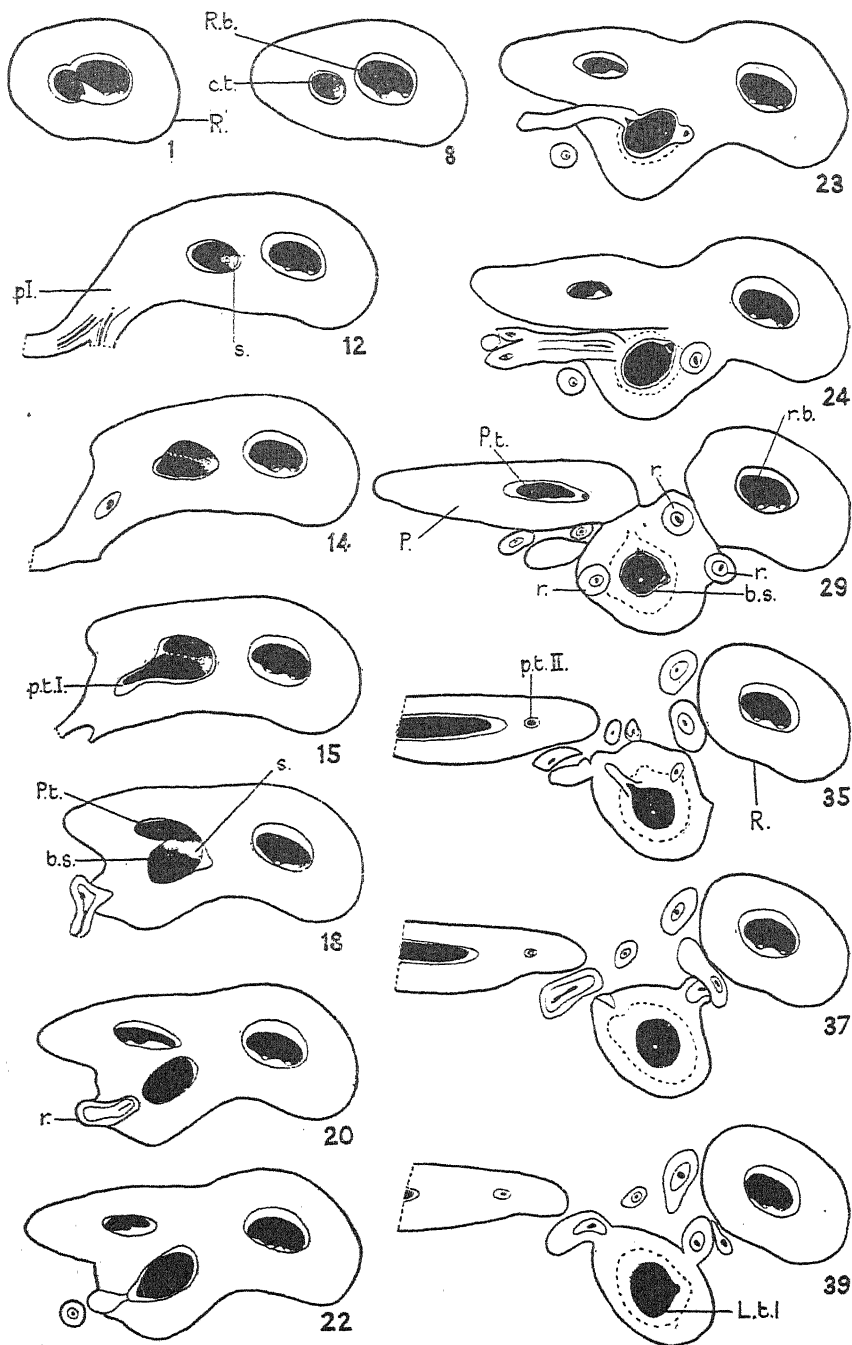
TEXT-FIG. 1. Camera-lucida drawings of the principal sections in a series through a leaf-borne bud of *B. hirsuta* Will. (Specimen A.) (× 6.) Numbers refer to the actual sections in the series. *R.* = rachis; *R.b.* = rachis bundle; *c.t.* = common trace; *s* = small tracheides; *pI* = first pinnule, *pII* = second pinnule; *p.t. I, p.t. II* = first and second pinnule traces; *P* = pinna, *P.t.* = pinna-trace; *b.s.* = bud-stele; *r* = root; *L.t. 1–L.t. 3* = first to third leaf-traces of bud; *L 1–L 5* = first to fifth leaves of bud.
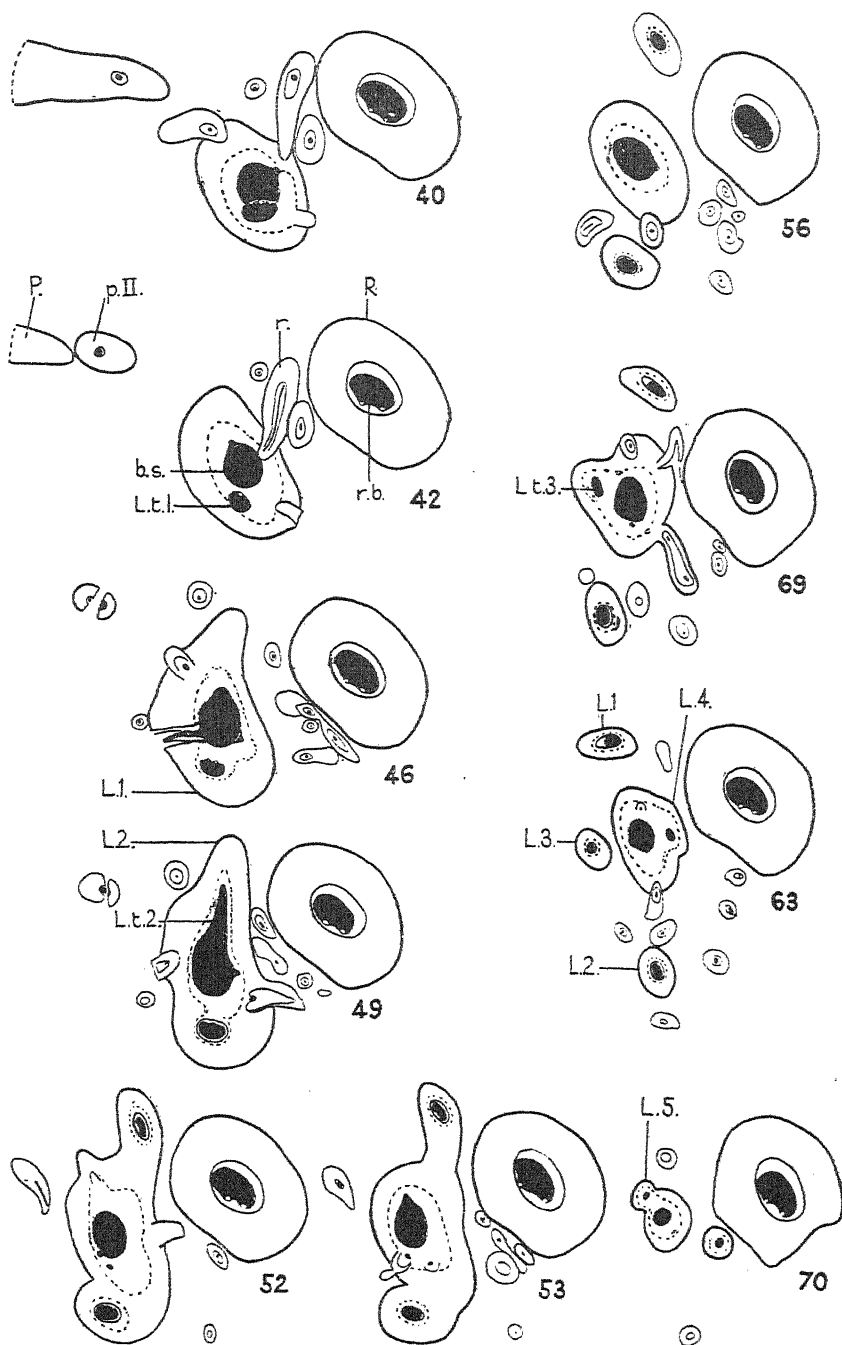
TEXT-FIG. 2. Continuation of Text-fig. 1. For description see Text-fig. 1.

in the lower region, and in addition, five small leaves are given off in spiral sequence.

After this summary account of the main features shown by the sections we may return to consider in detail the changes in the common trace as it separates from the bundle of the main rachis or petiole and passes to the pinna and bud. The initial condition seen in section No. 1 is shown on Pl. IV, Figs. 1 and 7. Here the common trace is just separating from the bundle of the main rachis and possesses a single group of small tracheides interpreted as protoxylem. In section No. 8 (Text-fig. 1 and Pl. IV, Fig. 8) the common trace has completely separated from the rachis bundle, which now shows only two adaxial teeth. This condition in the main rachis bundle is maintained throughout the rest of the sections. In section 12 the common trace is oriented slightly differently as compared with its position in the earlier sections. Thus the area of small tracheides now lies towards the rachis bundle rather than towards the adaxial surface. Moreover the common trace has become mesarch (Pl. IV, Fig. 9). The intermediate sections (e.g. Pl. IV, Fig. 8) show that this occurs by the protoxylem becoming gradually covered beneath the increasing number of smaller tracheides. Section 12 also shows the presence of the first pinnule, a branched exogenous appendage possessing two fine vascular strands. These two vascular strands are formed by the division of a single trace given off at a slightly higher level (section 15, Text-fig. 1). It is apparent that the pinnule was incomplete, since the distal region is either broken or decayed. The exogenous origin, branching, and general position rule out any possible confusion of this first pinnule with a root, nor can it possibly be interpreted as a petiole arising on the bud.

Section 15 (Text-fig. 1 and Pl. IV, Fig. 10) shows a preparation for division in the common trace. At this level both portions of the dividing trace are almost identical in appearance, except for the pinnule trace arising towards the adaxial side. Another small difference is the presence of a slightly larger number of small tracheides in the adaxial portion. A small cavity probably formed by the decay of thin-walled cells is present on the inside of the area occupied by the small tracheides. Above this level the two portions of the divided common trace gradually separate and with their separation proceeds an increasing differentiation.

In section 18 (Text-fig. 1) the base of the bud is clearly visible in cortical continuity with the pinna and main rachis. The common trace to the pinna and bud is shown in greater detail on Pl. IV, Fig. 11, and is seen to be now almost completely divided. The abaxial portion, which forms the pinna-trace, is composed chiefly of large metaxylem tracheides; the largest of these measures 0·16 mm. in diameter. In the adaxial portion, which represents the base of the cylindrical protostele of the bud, numerous small tracheides are present in the region towards the pinna. The remainder, however, is occupied by larger tracheides of almost the same size as the largest in the pinna-trace.

Above the level of section 18 a gradual separation of the main rachis, bud, and pinna takes place. Simultaneously all the tracheides of the bud-stele

become uniformly smaller in cross-section than those of the pinna; thus in section 29 (Pl. IV, Fig. 3) the tracheides in the pinna measure 0·13 mm. in maximum diameter and in the bud-stele 0·06 mm. In the pinna the xylem has a slightly tridentate appearance and the upper lobe becomes constricted off as the supply to the second pinnule borne on the upper side of the pinna. This second pinnule is shown separate from the pinna in section 42 (Pl. IV, Fig. 4), the pinna being merely represented at this level by an oblique slice through its broken end. The dimensions of this pinnule are 1·35 mm. and 0·8 mm. across its maximum and minimum diameters. The occurrence and position of both the first and second pinnules have been verified in a number of specimens.

Considering the bud and the changes which occur in its stele when followed through the series, in section 20 the bud-stele first appears distinct from the pinna-trace. It rapidly assumes the typical condition of a solid cylindrical protostele and the large tracheides characteristic of the stele in its lower region give place to uniformly smaller tracheides which, however, appear slightly narrower at the periphery and in the centre. In the latter region a group of tracheides arranged in a radial manner around two or three small elements appears to recur in successive sections. Although somewhat obscure in the lower region (e.g. Pl. IV, Fig. 12), this group appears to be continuous with the protoxylem associated with the first leaf-trace given off at a higher level. The portion of the metaxylem which separates as the first leaf-trace is distinguished by the slightly larger size of its tracheides, as seen in transverse section, and becomes apparent first in section 32. It is shown at the level of section 37 on Pl. IV, Fig. 13, where the maximum diameter of the tracheides in the normal part of the stem-stele is 0·054 mm. and in the leaf-trace region 0·072 mm. Although at least one other distinctive group of smaller xylem elements is present in the lower sections, as shown on Pl. IV, Fig. 12, no such group can be followed with certainty or shown to pass into any of the other leaf-traces. Hence in the camera-lucida drawings of Text-figs. 1 and 2 only a single protoxylem group is shown, although it cannot be said definitely that this was the only one present.

In this basal region of the bud numerous roots are borne on the stem and their endogenous origin may be seen in section 29 (Pl. IV, Fig. 3). At this level the stem has a maximum diameter of 2·5 mm. and the stele measures 0·9 mm. in cross-section. The cortex of the young stem is differentiated into two zones, an outer zone of thick-walled cells, and an inner thin-walled zone. The latter is much decayed and limited internally by a dark-coloured layer which possibly represents the remains of the endodermis. A layer of cells similar in position to the latter is figured by Scott (1920, p. 340) in the petiole. Between this layer and the xylem of the bud-stele is a narrow ring of decayed tissue presumably representing the phloem (Pl. IV, Fig. 12).

The first leaf-trace derived from the bud has already been referred to and is shown, after its separation from the bud-stele, on section 42 (Pl. IV, Fig. 4). During the departure it carries with it a single protoxylem group, which

becomes extended laterally on the inner face of the metaxylem bundle and then divides, giving rise to at least two, and possibly three, small tooth-like projections seen on Pl. IV, Fig. 14. On following the trace into the petiole the preservation deteriorates so that the entire structure of the young leaf could not be determined. It is possible to say, however, that the bundle does not show any rotation about its longitudinal axis.

Section 49 (Text-fig. 2 and Pl. IV, Fig. 15) shows the departure from the bud-stele of the second leaf-trace. It offers a different appearance from the first, but this is accounted for by the obliquity of the section. As a result of this obliquity the side of the bud on which the second petiole arises is cut a little higher than the opposite side on which the first petiole arises. It was expected that the oblique angle at which this second trace is cut might have revealed the presence of a protoxylem group seen unmistakably in longitudinal section. This would have helped to confirm the nature of the small group of narrow tracheides seen in the first leaf-trace. No such group of small tracheides, however, can be detected, either in this section or in the adjacent ones. This may be due to the inferior preservation of the tracheide walls, in consequence of which the pitting cannot be seen on even the largest metaxylem tracheides. What is apparent from the photograph, however, is that the average size of the tracheides in the leaf-trace is again slightly larger than in the stem-stele.

Section 58 (Pl. IV, Fig. 5) shows the origin of the third leaf-trace and also the first and second leaves now completely separate from the stem. These leaves are much smaller in transverse section than the stem of the bud on which they were borne.

The departure of the fourth leaf-trace from the bud-stele is shown on section 62 (Pl. IV, Fig. 6). Near the apex of the bud a fifth leaf-trace departs, though owing to the delicate nature of the tissue in this region it is poorly preserved and is seen only obscurely on section 67 (Pl. IV, Fig. 16). The general arrangement of these five leaf-traces is that of a two-fifths phyllotaxy. The diameters of the stem and stele markedly decrease in this apical region; the tracheides also are much narrower than at the base. This difference in size of the stele is evident from a comparison of Figs. 13 and 17 of Pl. IV. The measurements in the basal region as already given are: diameter of stem 2·5 mm., diameter of stele 0·9 mm., maximum diameter of tracheides 0·06 mm. At the level of section 70 the stem measures 1·26 mm. in diameter, the stele 0·36 mm., and the tracheides 0·036 mm. in diameter. Thus the diameter of the stem in the upper region is about one-half of that in the basal region, whilst its stele is about two-fifths of the diameter of the stele at the base. This reduction in relative size of the stele at the apex is not due merely to a diminution in size of the tracheides. Thus if the number of tracheides across any diameter of the stele is counted at the apex and at the base it is found to become smaller on nearing the apical region. The tissues of the actual apex of the bud are largely decayed, due, apparently, to their delicate nature.

(b) *The reconstruction.*

It is now possible to describe briefly the external appearance of the bud as deduced from the reconstruction shown diagrammatically in Text-fig. 3. It has been shown that in position the bud is adaxial to the leaf on which it was
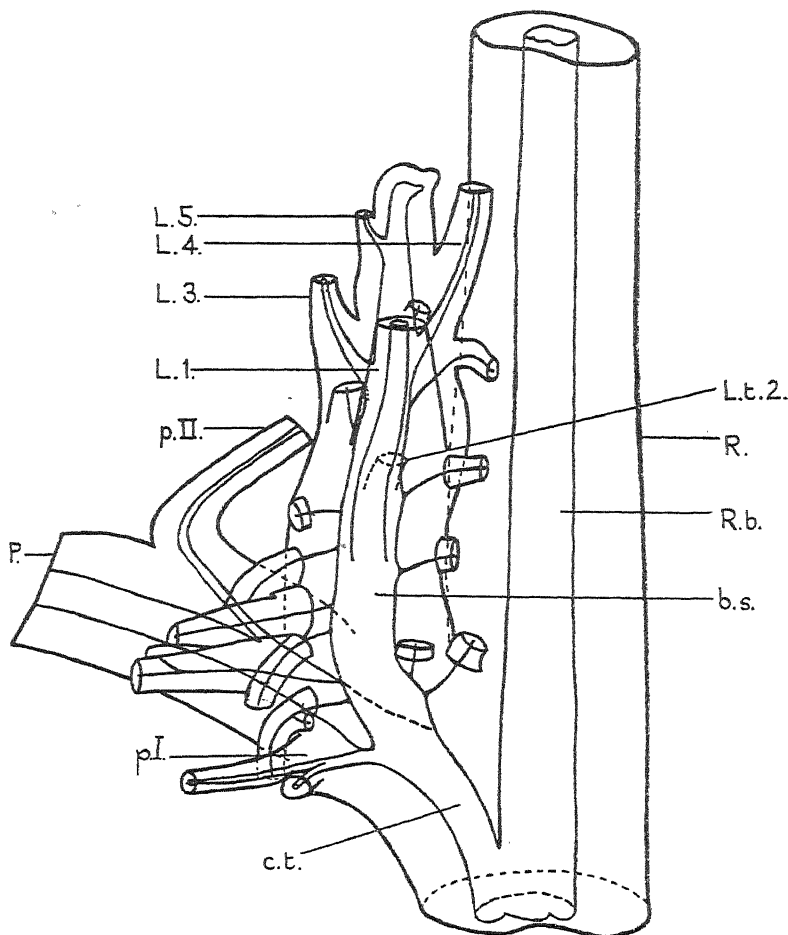
TEXT-FIG. 3. Diagrammatic reconstruction of bud and leaf fragment of *B. hirsuta* Will., seen from the adaxial side and showing the vascular structure. (Specimen A.) (× 8.) Abbreviations as for Text-fig. 1. N.B. Only the bases of the leaves are reconstructed; the remaining appendages not labelled are roots.

borne and situated at the base of a pinna. With it is associated the first pinnule borne on the pinna. The bud, however, is situated so close to the main petiole or rachis that its position may be described as subaxillary. The external form of the bud is that of a narrow erect cone. There is no apparent relationship between the position of the roots and of the leaves in this specimen. As is typical of the plant the bases of the leaves and surface of the stem, especially
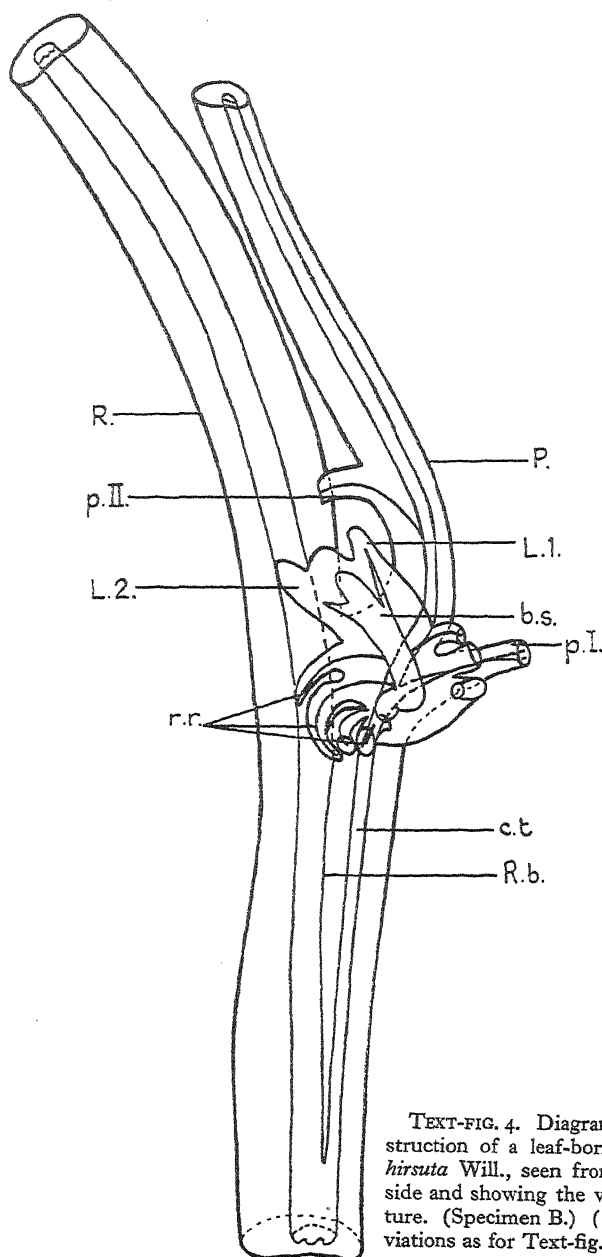
TEXT-FIG. 4. Diagrammatic recon-
struction of a leaf-borne bud of *B.
hirsuta* Will., seen from the adaxial
side and showing the vascular struc-
ture. (Specimen B.) (× 6.) Abbre-
viations as for Text-fig. 1.

at the apex, are clothed with numerous multicellular hairs. These hairs are
omitted from the reconstruction for the sake of clarity.

The bud measures 1 cm. in length and its maximum diameter is 2·5 mm.
The main petiole or rachis at the level of the first section in the series is 4 mm.
in maximum diameter, and the length of the portion examined was a little

over 2 cm. In this length the rachis only gave rise to the single pinna bearing the bud.

IV. SPECIMENS B AND C

On Text-figs. 4 and 5 diagrammatic reconstructions of portions of two leaves of *B. hirsuta* Will. are given, each showing a bud present at the base of
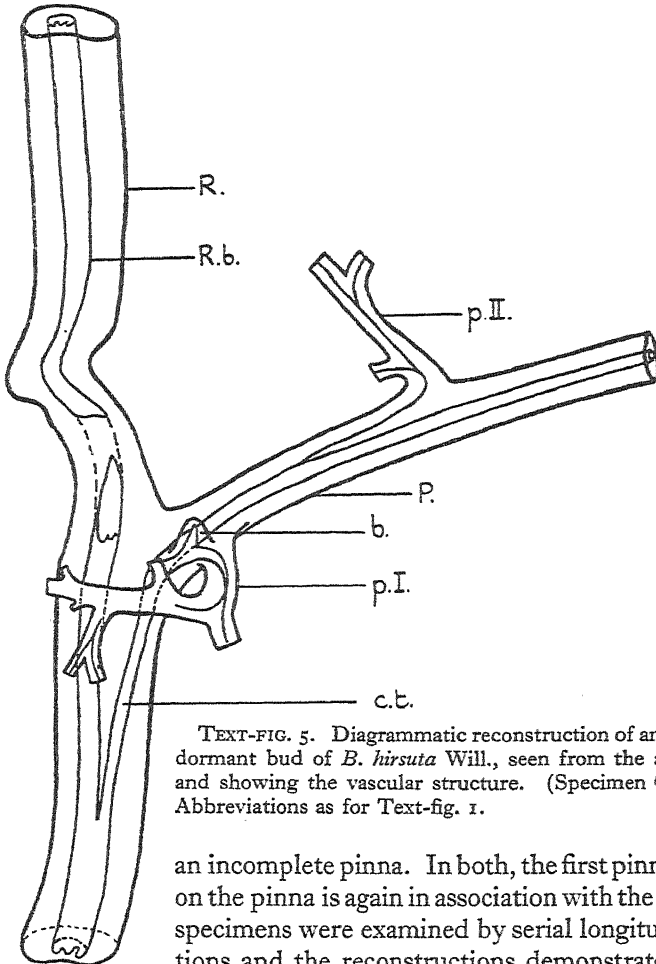
TEXT-FIG. 5. Diagrammatic reconstruction of an apparently dormant bud of *B. hirsuta* Will., seen from the adaxial side and showing the vascular structure. (Specimen C.) (× 6.) Abbreviations as for Text-fig. 1.

an incomplete pinna. In both, the first pinnule borne on the pinna is again in association with the bud. The specimens were examined by serial longitudinal sections and the reconstructions demonstrate that the relationship of parts is the same as in specimen A. They also serve to give some idea of the range in size and degree of development met with.

In specimen B (Text-fig. 4) the short stem of the bud is curved and bears two small leaves and numerous roots. The positions of the first and second pinnules on the pinna are exactly as in specimen A. The maximum diameter of the main petiole or rachis is 3 mm. and the total length examined was 6 cm., although this is not all shown on the figure.

Specimen C (Text-fig. 5) is of interest in that it illustrates how extremely

rudimentary the bud may be—only 1·6 mm. in length and apparently in a dormant condition. Thus there is no clear indication of either roots or leaves, though a definite vascular supply to the bud is present. Of further interest is the lateral alternate branching of both the first and second pinnules, without any certain indication of a lamina. The main rachis measures 2·1 mm. in maximum diameter, and the total length examined was 6 cm. In both specimens B and C the xylem strand of the rachis returns to the tridentate condition above the level of the bud, but in neither is there any indication of other pinnae. The curved nature of the bud-stem in specimen B is a feature which has frequently been seen in other specimens. In such buds the stem may be cut twice in the same section, giving a superficial resemblance to branching, though actually no branching of the stem has been seen.

V. Conclusion

It is not purposed at present to enter into a comprehensive discussion of the facts relating to the occurrence of these buds on the leaves of *B. hirsuta* Will. Certain points, however, do call for brief mention and comment. Thus, the question naturally arises as to whether similar buds occur on any other of the well-known species in this genus. To ascertain this, fragments of leaves of both *B. antiqua* Kidston and *B. ramosa* Will. have been examined with the result that one clear example of a similarly placed bud has been found in *B. antiqua* from Pettycur. Such buds have not been seen up to the present at the petiolar branchings of *B. ramosa*; further investigation on this point is desirable.

The general relative positions of main rachis, pinna, and bud are similar in all the examples of *B. hirsuta* investigated. Occasionally, however, the proportions in the division of the common trace to supply the pinna and bud show some variation. Thus in some specimens, the bud-stele attains its cylindrical form and full size before its separation from the pinna-trace. The result is that in such specimens the bud appears to arise directly on the main rachis, and the pinna bears a superficial resemblance to the first leaf of the bud. It is interesting that this is also very markedly the condition in the single bud of *B. antiqua* mentioned above.

No shoots have been met with which bore leaves equal in size to those on which the buds occur and most of the shoots examined have been proved to arise as buds. The larger petioles at the branching of which the buds described here are situated must have been borne on stems of a slightly larger size than any yet recorded.

The leaves borne by the buds were all very small and their leaf-bases did not swing round when leaving the stem (cf. Scott, 1920, p. 341). This may have possibly occurred, however, in the case of larger leaves. Nevertheless, in the light of the occurrence of the buds described, the possibility must be kept in mind that what may superficially appear to be a large petiole arising from a smaller stem and rotating about its long axis during departure may, on the

other hand, be interpreted in the opposite way as a leaf rachis bearing the shoot as a lateral bud.

It is, of course, impossible to say in describing these buds of *B. hirsuta* Will. how much of their growth took place either during or after the period when the leaf was still growing on the parent plant. Attention may be drawn, however, to the manner in which the bud-stem and its stele in specimen A diminish in diameter towards the apex. This feature may be an expression of arrest of growth and not merely the indication that the apical region of the bud is being approached.

Without entering into details it is interesting to recognize that buds on the leaves of some living leptosporangiate ferns occupy a similar position at the point of departure of a pinna from the rachis. It is of special interest to discover leaf-borne buds in this position in two species of Botryopteris representative of both the Lower and Upper Carboniferous periods.

The larger question of the morphological significance of these buds will not be dealt with at present.

SUMMARY

1. The occurrence of buds borne on the leaves of *Botryopteris hirsuta* Will. is placed on record and a description given of a single specimen supplemented by brief accounts of two other specimens.
2. All the buds examined have been situated adaxially and singly at the base of a pinna.
3. There is vascular continuity between the bud and the pinna.
4. The first pinnule borne on the pinna has become slightly adnate to the bud. A second pinnule is borne farther out on the pinna and on the upper surface; both pinnules may show alternate lateral branching.
5. In size the buds vary from very small structures about 1·6 mm. in length to well-developed young plants 1 cm. or more in length and bearing numerous roots and leaves.
6. The leaves borne on the bud conform to a two-fifths phyllotaxy. The diameter of their petioles is less than that of the bud-stem.
7. The tracheides of the leaf-trace are slightly but characteristically larger than those of the bud-stele.
8. Above the region of the bud the xylem of the main rachis regains its tridentate form.
9. The bud-stem and its stele may diminish considerably in diameter towards the apex.
10. A clear example of a similar leaf-borne bud is recorded in *Botryopteris antiqua* Kidston from Pettycur.

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EXPLANATION OF PLATE IV

Illustrating Mr. Long's article 'On the Occurrence of Buds on the Leaves of *Botryopteris hirsuta* Will'.

All figures refer to specimen A described in the text.

Fig. 1. Transverse section of relatively main petiole or rachis showing preparation for division. ($\times$ 8.) (Section No. 1.)

Fig. 2. Transverse section of main rachis showing the common trace to pinna and bud and the first pinnule. ($\times$ 8.) (Section No. 8.) *c.t.* = common trace; *pI* = first pinnule.

Fig. 3. Transverse section through main rachis, the pinna cut obliquely and the bud just separate. ($\times$ 8.) (Section No. 29.)

Fig. 4. Transverse section through main rachis and bud with broken tip of pinna and second pinnule cut obliquely. ($\times$ 8.) (Section No. 42.) *P* = pinna; *pII* = second pinnule.

Fig. 5. Transverse section of bud showing departure of third leaf-trace from bud-stele. ($\times$ 8.) (Section No. 58.) *L1–L3* = 1st, 2nd, and 3rd leaves of bud.

Fig. 6. Transverse section of bud showing the departure of the fourth leaf-trace from the bud-stele. ($\times$ 8.) (Section No. 62.) *L1–L4* = 1st to 4th leaves of bud.

Fig. 7. Transverse section of the common trace before separation from the main rachis bundle. ($\times$ 35.) (Section No. 1.)

Fig. 8. Transverse section of common trace after separation from the main rachis bundle. ($\times$ 35.) (Section No. 12.)

Fig. 9. Transverse section of common trace now apparently mesarch. ($\times$ 35.) (Section No. 12.)

Fig. 10. Transverse section of common trace preparing to divide and giving off the first pinnule trace. ($\times$ 35.) (Section No. 15.) *P.t.* = pinna-trace; *p.t.* = trace of first pinnule; *b.s.* = bud-stele.

Fig. 11. Transverse section of common trace almost completely divided. ($\times$ 35.) (Section No. 18.) *P.t.* = pinna-trace; *b.s.* = bud-stele.

Fig. 12. Transverse section of bud-stele in basal region. ($\times$ 35.) (Section No. 25.)

Fig. 13. Transverse section of bud-stele showing the first leaf-trace prior to its departure. ($\times$ 35.) (Section No. 37.) *px.* = protoxylem; *L.t.* 1 = first leaf-trace.

Fig. 14. Transverse section of bud-stele and first leaf-trace. ($\times$ 35.) (Section No. 42.)

Fig. 15. Transverse section of bud-stele with the second leaf-trace cut obliquely. ($\times$ 35.) (Section No. 49.)

Fig. 16. Transverse section of bud-stele near apex showing origin of fifth leaf-trace. ($\times$ 35.) (Section No. 67.) *L.t.* 5 = fifth leaf-trace.

Fig. 17. Transverse section of bud in apical region. ($\times$ 35.) (Section No. 70.)

7

p.t

8

P.t.

9

p.t

10

b.s.

12

P.t.

b.s.

11

p.x

L.t.1
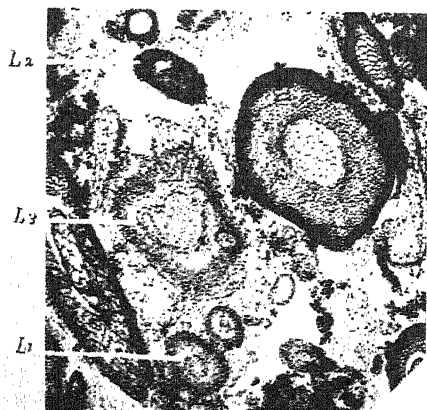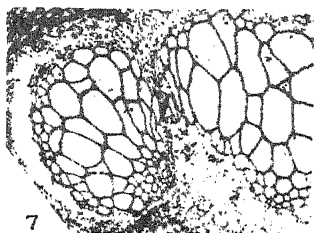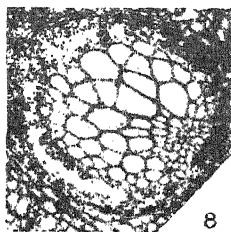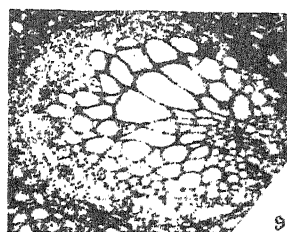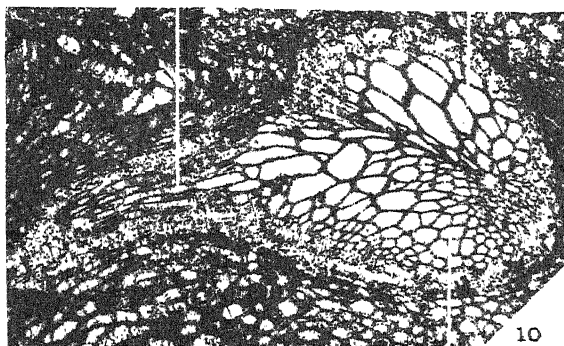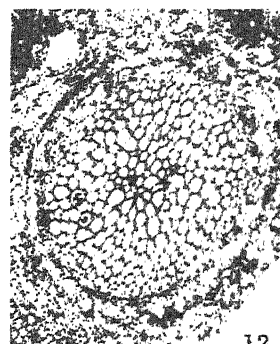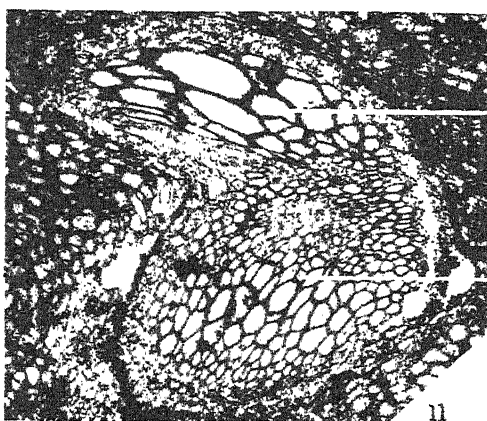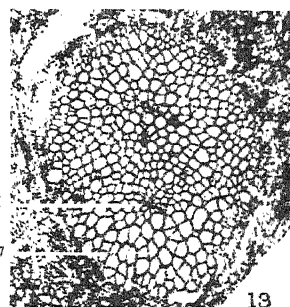
13

L.t.s
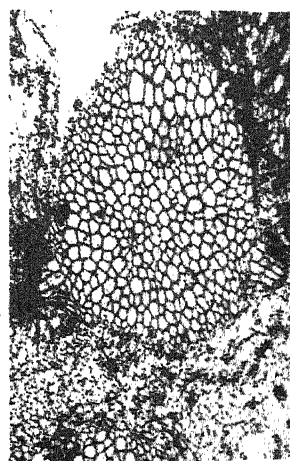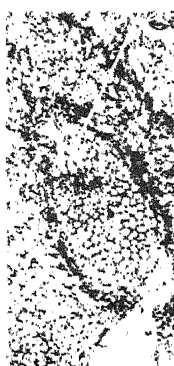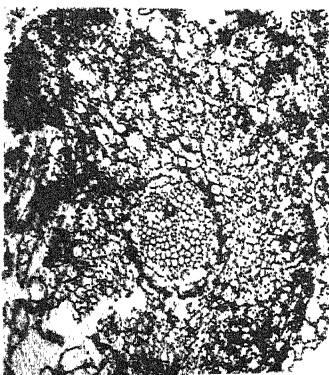
Studies on Foliar Hydration in the Cotton Plant

III. Preliminary Observations using the Pruning Method[1]

BY

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AND

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With two Figures in the Text

I. INTRODUCTION

IN a previous paper (Mason and Phillis, 1942) we recorded that discs punched from leaves and floated on salt solutions absorbed considerable amounts of salt and water. It was suggested that the hydration capacity of the protoplasmic proteins might be affected by salt in the same way that the hydration capacity of gelatin is affected by salt in the iso-electric region (cf. Jordan and Shore, 1938). Certain limitations of the gelatin analogy were stressed and the possibility indicated that metabolic factors were involved.

We have frequently noticed that a reduction in the number of leaves on a plant is followed by a marked increase in the size of the leaves that remain (cf. Gardner, Bradford, and Hooker, 1922). This increase in the size of the leaf takes place irrespective of the age of the leaf. As this increased size of the leaf is evidently largely due to an increase in its water content, it occurred to us that this afforded another method of exploring the factors controlling foliar

[1] Paper No. 30 from the Physiological Department of the Cotton Research Station, Trinidad.

hydration. As no meristems are present in the leaf,[1] it seems clear that an increase in the water content of the leaf must involve an increase in the amount of water in the individual cells. Two experiments involving a reduction in the number of leaves are described in this paper.

II. METHODS

Fresh weight, dry weight, and total-N were determined on each sample. On the expressed sap, crystalloid-N, depression of freezing-point, and specific conductivity were also determined. In the first experiment total sugar concentration and pH of sap were measured. The specific conductivity (at $0°$ C.) was measured on sap diluted 10 times and the dilution was allowed for in presenting the results. Protein-N was taken to be the difference between total-N and crystalloid-N.

III. THE EFFECT OF PARTIAL DEFOLIATION ON LEAVES OF PLANTS SUPPLIED WITH VARYING AMOUNTS OF NITROGEN, POTASSIUM, AND PHOSPHORUS (*Experiment 1*)

A. *Procedure.*

Plants were grown in sand culture in a greenhouse which was kept humid by repeated spraying. There were 6 nutrient treatments, as shown in Table I. When the plants were 7 weeks old, the leaves on nodes 5, 6, and 7 from the apex were marked with wool. The plants of each nutrient treatment were subdivided into two groups as follows:

Group 1. 'Normal.' No treatment.

Group 2. 'Pruned.' All leaves except marked ones as well as all buds and branches were removed. Approximately 80 per cent. of the leaves were removed.

After 8 days the marked leaves were collected from each of the 12 groups of plants. There were 6 plants per sample and 2 samples from each group. A sample thus consisted of 18 leaves.

TABLE I

Nutrient Solutions employed for Growth of Plants of Experiment 1

	Supply (p.p.m.)		
	Nitrogen.	Potassium.	Phosphorus.
Low nitrogen	100	100	50
High ,,	300	100	50
Low potassium	200	80	50
High ,,	200	160	50
Low phosphorus	200	100	20
High ,,	200	100	320

All treatments were supplied with calcium and magnesium (100 p.p.m. each): iron and trace elements were supplied as usual.

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[1] The cells of the spongy mesophyll immediately abutting on the lower epidermis are, however, transformed into a palisade.

B. *Results.*

In Table II we show the weights of water, dry weight, salt, and protein-N per sample of 18 leaves in the 'Normal' and 'Pruned' plants. The percentage increases of the 'Pruned' over the 'Normal' plants are also shown. It will be seen that pruning has increased the water contents of the leaves; these increases range from 30 per cent. (Low Nitrogen) to 41 per cent. (High Phosphorus). The increases in dry weight ranged from 28 per cent. (Low Nitrogen) to 38 per cent. (High Phosphorus); those of salt from 25 per cent. (High Potassium) to 43 per cent. (Low Potassium), and those of protein-N from 37 per cent. (High Nitrogen) to 49 per cent. (Low Nitrogen and High Phosphorus).

TABLE II

Water, Dry Weight, Salt, and Protein-N Contents of 18 Leaves from Normal and from Pruned Plants together with Relative Changes due to Pruning

		Water.	Rel. change.	D.Wt.	Rel. change.	Salt.	Rel. change.	Protein-N.	Rel. change.
Low Potassium	'Normal'	56·68	—	11·61	—	8·50	—	0·484	—
	'Pruned'	79·09	40	15·30	32	12·18	43	0·687	42
High Potassium	'Normal'	62·83	—	12·02	—	10·74	—	0·485	—
	'Pruned'	82·43	31	15·64	30	13·44	25	0·699	44
Low Nitrogen	'Normal'	50·20	—	9·25	—	7·83	—	0·325	—
	'Pruned'	65·34	30	11·80	28	10·32	32	0·485	49
High Nitrogen	'Normal'	61·88	—	12·08	—	9·53	—	0·517	—
	'Pruned'	83·87	36	15·95	32	13·42	41	0·707	37
Low Phosphorus	'Normal'	54·91	—	11·39	—	8·73	—	0·477	—
	'Pruned'	75·22	37	14·77	30	12·11	39	0·663	39
High Phosphorus	'Normal'	58·68	—	11·03	—	9·45	—	0·441	—
	'Pruned'	82·92	41	15·26	38	13·18	39	0·655	49

TABLE III

pH, Depression of Freezing-point, Specific Conductivity ($C \times 10^3$) and Total Sugar Gm. per 100 Gm. Water of Saps, from Leaves of Normal and Pruned Plants together with Relative Changes due to Pruning

		pH	Rel. change.	Dep. of F.P.	Rel. change.	Sp. cond.	Rel. change.	T. sugar.	Rel. change.
Low Potassium	'Normal'	5·15	—	0·82° C.	—	15·0	—	0·437	—
	'Pruned'	4·98	−3·3	0·83° C.	1·3	15·4	2·3	0·212	−51·5
High Potassium	'Normal'	5·32	—	0·86° C.	—	17·1	—	0·182	—
	'Pruned'	5·04	−5·2	0·85° C.	−1·1	16·3	−4·7	0·207	13·7
Low Nitrogen	'Normal'	5·08	—	0·80° C.	—	15·6	—	0·196	—
	'Pruned'	4·97	−2·1	0·82° C.	2·5	15·8	1·3	0·195	−0·5
High Nitrogen	'Normal'	5·15	—	0·80° C.	—	15·4	—	0·284	—
	'Pruned'	5·02	−2·5	0·85° C.	6·3	16·0	3·9	0·212	−25·4
Low Phosphorus	'Normal'	5·39	—	0·80° C.	—	15·9	—	0·235	—
	'Pruned'	5·16	−4·3	0·87° C.	8·8	16·1	1·3	0·217	−7·7
High Phosphorus	'Normal'	4·88	—	0·86° C.	—	16·1	—	0·197	—
	'Pruned'	4·80	−1·6	0·86° C.	0·0	15·9	−1·2	0·205	4·1

The freezing-point depressions, specific conductivities, pH, and total sugar concentrations in the saps of the 12 groups of plants are shown in Table III. The relative changes due to pruning are also shown in the table. The changes in freezing-point depression as a result of pruning were small, ranging from an increase of 9 per cent. (Low Phosphorus) to a decrease of 1

per cent. (High Potassium). The results do not suggest that a change in the osmotic pressure of the sap can have been responsible for the increased water contents of the leaves of the 'Pruned' groups. The changes in specific conductivity were also small, ranging from an increase of 4 per cent. (High Nitrogen) to a decrease of 5 per cent. (High Potassium). Pruning has consistently caused a slight drop in pH. It will be noticed that the pH of the sap of the High Phosphorus group is markedly less than that of any other treatment. Pruning has tended to bring sugars to a constant level in all groups, high values in the 'Normal' groups being reduced and low ones increased.

TABLE IV

Correlation Coefficients between Relative Changes in Water and those in Dry Weight, Salt, Protein-N, Depression of Freezing-point, and Specific Conductivity

	Dry weight.	Salt.	Protein-N.	Dep. of F.P.	Sp. cond.
$r =$	$+0.756$	$+0.837$	-0.170	-0.024	$+0.301$

   The correlation coefficients between the relative changes in water and those in dry weight, salt, protein-N, depression of freezing-point, and specific conductivities (see Tables II and III) are shown in Table IV. Significant (5 per cent. level) correlations are shown in italics. It will be seen that water and salt show a positive and significant correlation. The correlation coefficient between water and conductivity is, however, small and not significant, so that the strength of the correlation between salt and water is due largely to the fact that salt is calculated from conductivity × water. From the data it is impossible to decide on the cause of the increase in water uptake due to defoliation.

   Partial defoliation might have reduced the water strain in the leaves of the 'Pruned' groups and so increased the amounts of water in them. It might also, as a result of increased transpiration from the remaining leaves and/or an increased concentration of salts in the transpiration current, have caused a greater quantity of salts to enter the leaves of the 'Pruned' groups. Thus, defoliation might increase salts and water quite independently of one another. The increased salt uptake, which would include both nutrient and non-nutrient salts, might of course lead to the formation of more (anhydrous) protoplasm. Thus, the water content of the leaf might also have been increased either by an increase in the amount of protoplasm or by an increase in its hydration capacity. In any case, strong correlations between salt and water increases on pruning would be expected. It will be obvious that if pruning experiments are to be used in investigations on hydration, precautions must be taken to avoid differences in water strain and in nutrition.

IV. THE EFFECT OF VARYING THE CONCENTRATION OF THE SOLUTION AROUND
     THE ROOTS OF PARTIALLY DEFOLIATED PLANTS (*Experiment 2*)

   In this experiment we have eliminated the water strain factor by using *only* partially defoliated plants, and the nutrient factor by supplying the roots with calcium chloride solutions rather than a full nutrient solution.

A. *Procedure.*

The plants were grown in water culture and were supplied with our standard nutrient solution. When the plants were 9 weeks old the leaves on nodes 6, 7, and 8 from the apex were marked with wool. All other leaves as well as buds and fruiting branches were then removed. On the following day an initial collection was made of marked leaves. The remaining plants were then subdivided into 4 groups and treated as follows:

Group 1.   Culture solution replaced by water.
  ,,   2.   ,,   ,,   ,, $M/200$ calcium chloride.
  ,,   3.   ,,   ,,   ,, $M/100$,,   ,,
  ,,   4.   ,,   ,,   ,, $M/50$,,   ,,

Collections of marked leaves were made from each of these groups after 9, 20, and 27 days. At each collection there were 2 samples of 9 plants each from each group. A sample thus consisted of 27 leaves. The water and calcium chloride solutions around the roots were changed weekly. Freezing-point depressions of the sap were not determined in this experiment.

B. *Results.*

The changes in water and specific conductivity are shown in Fig. 1. The results for water are expressed on the sample basis, and both water and conductivity are expressed as percentages of the values at the initial collection. Both are plotted against time.

It will be seen that in the calcium chloride treatments (groups 2, 3, and 4) water increased throughout the duration of the experiment, while the increase in the water content of the water treatment (group 1) was small and not significant. It will also be noticed that the increase in water was greatest in the most concentrated and least in the most dilute solution. To sum up, *partial defoliation* per se *does not lead to an increased uptake of water by the leaves remaining on the plant. When, however, calcium chloride solutions replace water around the roots, the uptake of water is proportional to the concentration of calcium chloride in the solution. Leaves still on the plant, therefore, react to calcium chloride solutions in the same way as discs punched from leaves.*

The changes in conductivity do not call for much comment as they are very similar to those for water. The relative changes are, however, on the whole rather bigger than those for water. The water treatment shows an unexpected increase of about 20 per cent. As there was at the same time a small increase in water, it is clear that the amount of salt in the leaves was increased. This may have been due to leakage of salts into the transpiration current or to traces of salt in the water around the roots.

The relationship between conductivity and water is shown in Fig. 2. On the left of the figure the relative changes in water are plotted against the specific conductivities of the sap. All the results are shown in the figure. The results for each collection are connected by lines. The whole forms a curve in which at low conductivities relatively big changes in conductivity are associated

with relatively small changes in water, while at high conductivities the reverse obtains. The increases in conductivity are presumably due to calcium chloride since this was the only salt present in the water around the roots.
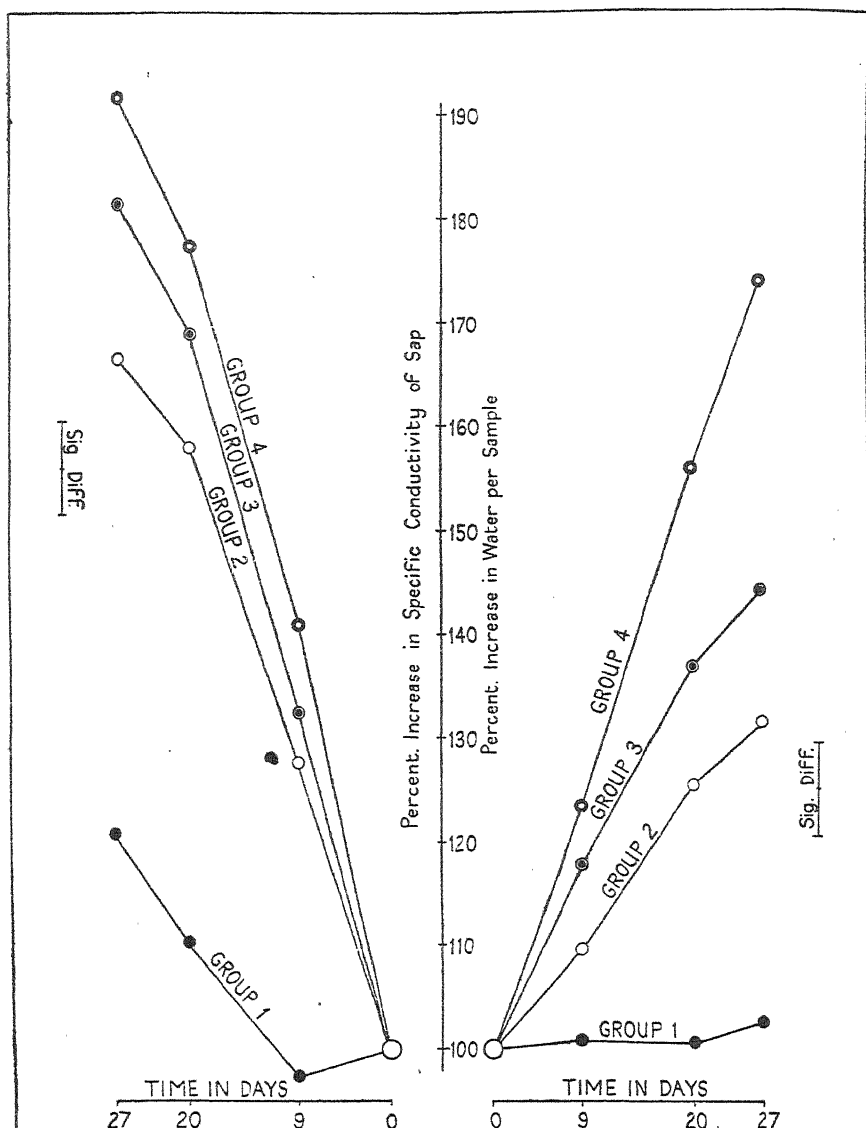
FIG. 1. Relative changes in water (right) and in specific conductivity (left) plotted against time. Water is expressed on the sample basis.

There are evidently two factors at work. This is shown by comparing the results for collection 2 with those of collection 3. For each collection there is a concentration effect similar to that shown by the whole series of data, viz. as
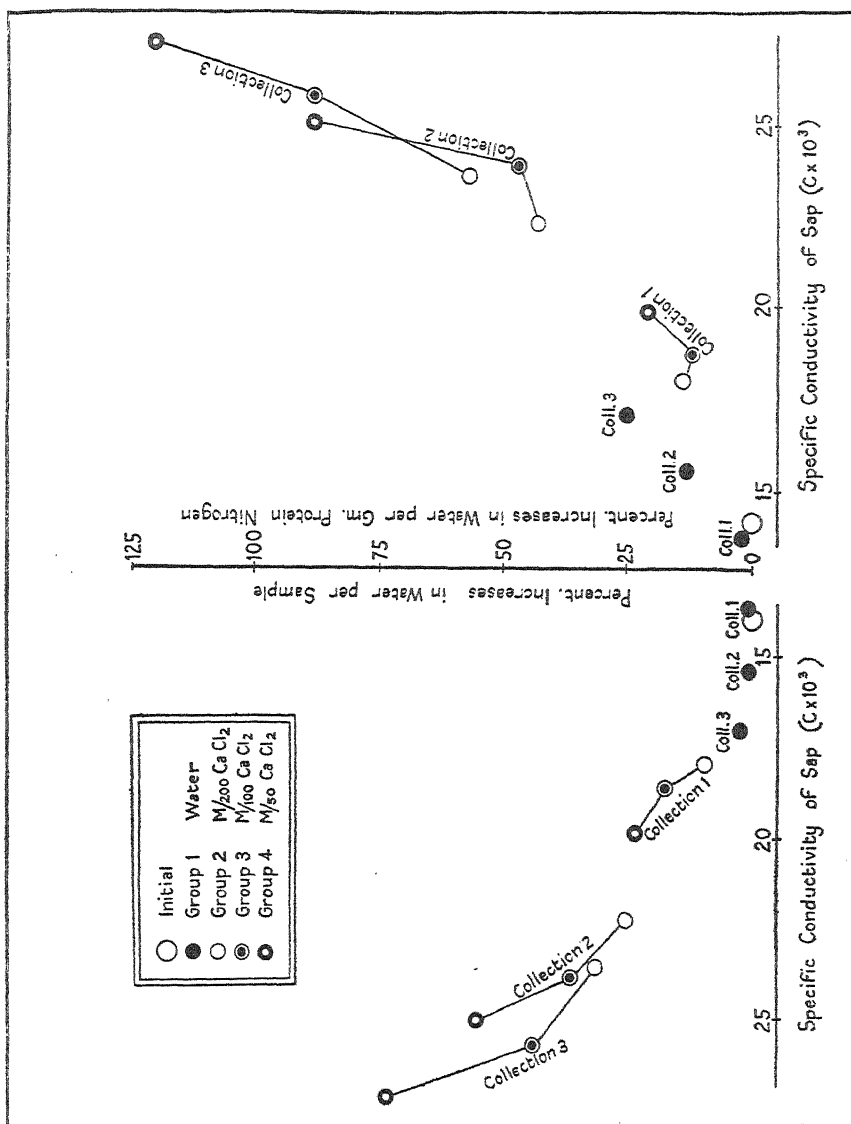
FIG. 2. Relative changes in water plotted against specific conductivity of sap. Water is expressed on the sample basis (left) and on the protein basis (right).

the concentration of salt increases, the increments in conductivity are accompanied by larger and larger increments in water. There is also a much smaller time effect which expresses itself in the reverse way, viz. as time advances a higher salt concentration is required to bring about a given change in water.

As there was a continuous loss of protein from the leaves throughout the duration of the experiment, the possibility arises that the time effect might have been due to this loss. The largest loss of protein amounted to 24 per cent. We have accordingly expressed water in terms of protein (i.e. water per gm. protein) and show (Fig. 2, right) the relative changes in water expressed in this way plotted against the conductivity of the sap. It will be seen that the differences between the results for collections 2 and 3 practically disappear.

V. Discussion

It would seem that the pruning method described in experiment 2 of the present paper may prove useful in experimentally manipulating the water content of the leaf. It may be asked, why not, instead of pruning, merely increase the concentration of salts around the roots? In experiment 1 we actually doubled the concentration of salts in the nutrient solution without partially defoliating the plants. The results of this treatment, which were not reported, were as follows: Doubling the concentration of the salts without pruning increased the average water content of the leaves by only 7 per cent., while pruning without altering the concentration of the salts increased the average water content by 37 per cent.

The experiments reported in the present paper, like those reported in our previous paper using the disc culture method, are of course purely exploratory. There is, however, a striking parallelism between the results obtained by the two methods. Thus, in the second experiment using discs and the first experiment using pruned plants, full nutrient solutions were used, and in both the increases in water were accompanied by little or no change in the specific conductivity of the sap. As both essential and non-essential elements were supplied, we have suggested that the uptake of water may have been due to an increase in the amount of protoplasm as well as to an increase in its hydration capacity due to the increase in salt.

In the third experiment using discs and the second experiment using pruned plants, only calcium chloride was supplied. In both experiments the increases in water content were accompanied by marked increases in the specific conductivity of the sap. As no nutrient elements were supplied, we suggest that the uptake of water was due to an increase in the hydration capacity of the protoplasm.

Our reasons for rejecting the classical osmotic theory of the cell's water relations are briefly as follows: Much of the cell's volume in the cotton leaf appears to be occupied by protoplasm and the larger part of the water in the cell (approximately two-thirds) is present in the protoplasm (cf. Mason and Phillis, 1939). The small central vacuole has only a low solute concentration

(22–23 atmospheres osmotic pressure), but solutions of much higher concentration than this are required to cause plasmolysis. Tissues from which water has been expressed by pressure can reabsorb water from solutions of much higher concentration than the vacuole. It seems clear that osmotic regulation between the vacuole and an external solution cannot be a factor of much importance in regulating the water content of the whole cell. Further, in our disc investigations we found that the depression of freezing-point of the expressed sap might increase while water content was diminishing. Assuming depression of freezing-point to be an adequate measure of concentration, it would seem that the *total solute concentration* cannot be a factor regulating water as required by the osmotic theory. Again, we have so far failed to obtain disc swelling with non-electrolytes. For all these reasons we have been compelled to reject osmotic regulation of the vacuole in the classical sense as the cause of water regulation in the leaf.

This conclusion receives support from the type of relationship between water content and conductivity shown in Fig. 2. If the calcium chloride were to function by changing the osmotic pressure of the vacuole, then it would be anticipated that the water increments would gradually diminish as the conductivity increased. Actually the reverse occurred. It should be added that this is also true of the disc experiment in which calcium chloride was used.

The relationship between water and conductivity is also the reverse of what would be expected if calcium chloride functioned as neutral salts do when they influence the hydration capacity of gelatin in the region of its iso-electric point. The relationship operating in the leaf between sap conductivity and water is not, as far as we know, duplicated by any known physical mechanism.

The data are not, however, sufficiently extensive to determine the mathematical nature of this relationship, but cursory inspection of the results shown in Fig. 2 suggests that a limiting value may be reached at which water uptake proceeds without change in conductivity. Some breakdown in protoplasmic structure may be involved. It should be added that the results shown in Fig. 2 are not materially altered if the results for conductivity are considered in terms of equivalents of calcium chloride. Nor is the relationship significantly affected if the conductivities are corrected for the back-pull due to the calcium chloride in the solution around the roots.

The effect of the protein correction shown on the right of Fig. 2 in eliminating the time effect shown on the left of the figure suggests that protein, at least under conditions of protein starvation, may be a useful guide to the amount of anhydrous protoplasm present in the leaf.

In our previous paper using the disc method, we were led to conclude that the amount of salt rather than the conductivity of the sap was the factor affecting water. A more critical examination of the results on which this conclusion was based has inclined us to the view that the evidence at present available does not enable us to decide whether the conductivity of the sap or the amount of salt is the more important.

VI. Summary

1. Advantage has been taken of the known fact that defoliation influences the size and water content of the leaves that remain on the plant to investigate further the relation between electrolytes and hydration.

2. An experiment is described in which the effects of partial defoliation upon the remaining leaves of a plant supplied with a full nutrient solution were investigated. It was found that pruning caused increases in the water, dry weight, and protein contents, but only small changes in depression of freezing-point and conductivity of the sap. Difficulties in the interpretation of the results of experiments of this type are pointed out.

3. A second experiment is described in which the confusing effects of variable water strain and of variable nutrition were removed. Plants grown in a full nutrient solution were partially defoliated and transferred to calcium chloride solutions and subsequent changes in the remaining leaves noted. Under these conditions, the uptake of water by the leaves was proportional to the increase in calcium chloride content.

4. It is pointed out that the behaviour of leaves on pruned plants supplied with a full nutrient solution is similar to the behaviour of *discs* floated on a full nutrient solution and that *leaves* on a pruned plant supplied with calcium chloride solution behave like *discs* floated on such solutions.

5. It is not certain whether the *total amount* of salt or its *concentration* in the sap is the more important factor controlling hydration. Before this matter can be settled, much more must be known of the manner in which salts act in controlling hydration.

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Studies on Foliar Hydration in the Cotton Plant

IV. The Influence of Composition and Concentration of Nutrient Solution[1]

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With four Figures in the Text

I. INTRODUCTION

IN two recent papers (Mason and Phillis, 1942 *a*; Phillis and Mason, 1943) it was shown that the water content of the tissues of the leaf can be experimentally manipulated in a number of different ways. In the first paper use was made of discs punched from leaves and floated on salt solutions in light. In the second, use was made of the fact that partial defoliation increases the water content of the leaves left on the plant. The results for the discs and the leaves on partially defoliated plants were very similar. It was found that when full nutrient solutions were used, increases in the amount of water in the tissues occurred without any marked change in the specific conductivity of the expressed sap. It was suggested that in this type of experiment the uptake of water was determined by an increase in the amount of protoplasm rather than

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[1] Paper No. 31 from the Physiological Department of the Cotton Research Station, Trinidad.

by an increase in its hydration capacity. When solutions of calcium chloride were used in place of full nutrient solutions, it was found that the increase in the amount of water in the tissues was accompanied by a comparable increase in the specific conductivity of the expressed sap. It was suggested that the uptake of water in this type of experiment was due to an increase in the hydration capacity of protoplasm as a result of the increase in the concentration of electrolytes. The nature of the mechanism whereby the water content of the cell is influenced by electrolytes is still quite unknown. Apparently it is not osmotic (cf. Mason and Phillis, 1939, 1942 *a*; Phillis and Mason, 1943), and it has consequently been suggested that electrolytes in some way influence the hydration capacity of the protoplasm or of some constituent of protoplasm.

The object of the experiments reported in the present paper was to ascertain how far the conclusions drawn from our work on foliar discs and partially defoliated plants were applicable to leaves on plants *grown* with a variety of nutrient solutions. In the first experiment the *composition* rather than the *concentration* of the nutrient solution was varied. The nitrogen supply was kept constant, but potassium, calcium, sodium, and phosphorus were varied over wide limits. In the second experiment there were two sections, one with constant nitrogen supply and the other with variable nitrogen supply. In both sections the concentration in the nutrient solutions ranged from half to five times that of our standard nutrient solution. This experiment was concerned with changes in *concentration* rather than *composition*. In the third experiment the supply of nitrogen was varied, while all other nutrients were kept at a constant level.

II. BASIS OF EXPRESSION

We have used the word hydration in the title of this series of papers. The word hydration implies *combination* with water. Recent work on cotton leaves (Mason and Phillis, 1939; Phillis and Mason, 1941) indicates that normally about two-thirds of the water in the leaf is present in the protoplasm in such a form that it cannot be separated by mechanical pressure unless the protoplasm is first injured. In fact, the water present in protoplasm does not appear to be free water but to be in some form of combination with the non-aqueous constituents. The water in the vacuole is probably almost entirely free water, but unpublished results show that this water is in dynamic equilibrium with the cytoplasmic water.

The salt hypothesis of foliar hydration postulates that the hydration capacity of protoplasm can be controlled through its salt content. It is not yet clear whether the effective salt content is the concentration in the sap or the total amount present. In the present paper we consider *primarily* the concentration in the sap, as the total amount present is derived from both conductivity of sap and weight of water. The best basis to use in discussing the changes in the hydration capacity of protoplasm would be anhydrous protoplasm, but we cannot determine this. We can determine what we believe to be one of the major components of anhydrous protoplasm—protein, but here

again there is the difficulty that we do not know whether all the protein in the cell is utilized in protoplasm, nor do we know how constant is the proportion of protein in the non-aqueous part of protoplasm. If there is storage protein or if anhydrous protoplasm contains a variable proportion of protein, protein would be an imperfect measure of protoplasm.

In our previous papers on the influence of electrolytes on hydration we used the sample basis. This procedure is justified if the amount of anhydrous protoplasm remains relatively constant, as it may well do in a disc experiment in which a non-nutrient solution such as calcium chloride is used. When comparing leaves of plants which have been *grown* under different nutrient treatments, it is probable that there will be different amounts of anhydrous protoplasm in the leaves and so for comparison of hydration capacity the use of the sample basis would not be justified.

The plant physiologist is accustomed to express water in terms of dry weight. As a considerable part of the dry weight consists of non-protoplasmic materials (e.g. starch and cellulose) with a relatively low hydration capacity, dry weight would not appear to be a very adequate basis.

To sum up, we suggest that the weight of water in the leaf is determined by two factors (cf. MacDougall, 1920). The first is the weight of anhydrous protoplasm. This we shall term the 'Bulk Factor'. As we cannot determine the weight of anhydrous protoplasm, we employ both the dry weight and the weight of protein as *estimates* of its amount. The second factor controlling the weight of water in the leaf is assumed to be the hydration capacity of anhydrous protoplasm. In the present paper our main concern is to find out how the hydration capacity of the protoplasm is influenced by the concentration of electrolytes in the sap and we use water per 100 gm. dry weight and per gm. protein-N as estimates of the hydration capacity of the protoplasm.

III. Methods

The following weights were determined on each sample: dry weight of whole plant, fresh weight and dry weight of leaf. Total-N was determined on the dried leaf. On sap expressed from the leaf the concentration of crystalloid-N, the freezing-point depression, and the specific conductivity at $0°$ C. were determined. The sap was diluted 10 times for the measurement of the conductivity and the results multiplied by 10 to allow for this dilution. From these determinations, estimates were made of the weights of water, protein, and salt. The salt estimate was obtained from conductivity $\times$ the weight of water. The freezing-point depression of the sap is assumed to be an index of osmotic pressure. In experiment 3, freezing-point depressions were not made.

IV. Variation in Composition (*Experiment 1*)

A. *Procedure.*

This experiment is concerned with the effects of variations in the composition of the nutrient solution. Nitrogen was kept constant at 400 p.p.m., but

potassium, calcium, sodium, and phosphorus were varied over wide limits. There were in all 33 treatments, which are detailed in Table I. The freezing-point depressions of these solutions ranged from 0·13° C. to 0·33° C. Thus, in addition to wide variation in composition, there were, of course, also variations in concentration.

The plants were grown in sand culture in a greenhouse. The sand was leached and supplied with fresh nutrient solution weekly. After the third week, daily illumination was cut down from normal (approx. 13 hours) to 6 hours, in order to minimize the size effect (cf. Mason and Phillis, 1942 *a* The plants were collected when 7 weeks old.

<div align="center">TABLE I</div>

Potassium, Calcium, Sodium, and Phosphorus Contents (p.p.m.) of Nutrient Solutions, numbered 1–33

Sodium (p.p.m.)	1000	200	0	1000	0	
Calcium (p.p.m.)	25	100	500	500	25	
500 p.p.m. ⎫	1	2	3	28	—	⎫ 500 p.p.m.
100 p.p.m. ⎬ phosphorus	4	5	6	29	—	⎬ potassium
12·5 p.p.m. ⎭	7	8	9	30	—	⎭
500 p.p.m. ⎫	10	11	12	—	—	⎫ 100 p.p.m.
100 p.p.m. ⎬ phosphorus	13	14	15	—	—	⎬ potassium
12·5 p.p.m. ⎭	16	17	18	—	—	⎭
500 p.p.m. ⎫	19	20	21	—	31	⎫ 25 p.p.m.
100 p.p.m. ⎬ phosphorus	22	23	24	—	32	⎬ potassium
12·5 p.p.m. ⎭	25	26	27	—	33	⎭

B. *Results.*

The values for dry weight, water, and conductivity in the lamina for the 33 treatments are shown in Fig. 1. The results are expressed as percentages of the mean values. To simplify presentation, the points are arranged in order of increasing dry weight. The numbers of the treatments (see Table I) are shown in the figure. Protein changes (not shown) were very similar to those in dry weight ($r = +0.91$).[1] Changes in the depression of the freezing-point of the expressed sap (not shown) were highly correlated with changes in conductivity ($r = +0.92$). Reference to Fig. 1 will show that water showed a similar *trend* to dry weight, while conductivity did not. This is confirmed by a significant correlation between water and dry weight on the sample basis ($r = +0.71$), and by the absence of any correlation between water on the sample basis and conductivity ($r = -0.02$). Figure 1 shows, however, that there was a marked similarity in *pattern* between water and conductivity, and in fact, when the trend in water is removed by expressing it in terms of dry weight, there is a significant positive correlation between conductivity and water per 100 gm. dry weight ($r = +0.48$). Moreover, the relative changes in the two variables are of the same order (coefficient of variability for water per 100 gm. dry

[1] Significant correlations (5 per cent. level) are in heavy type.
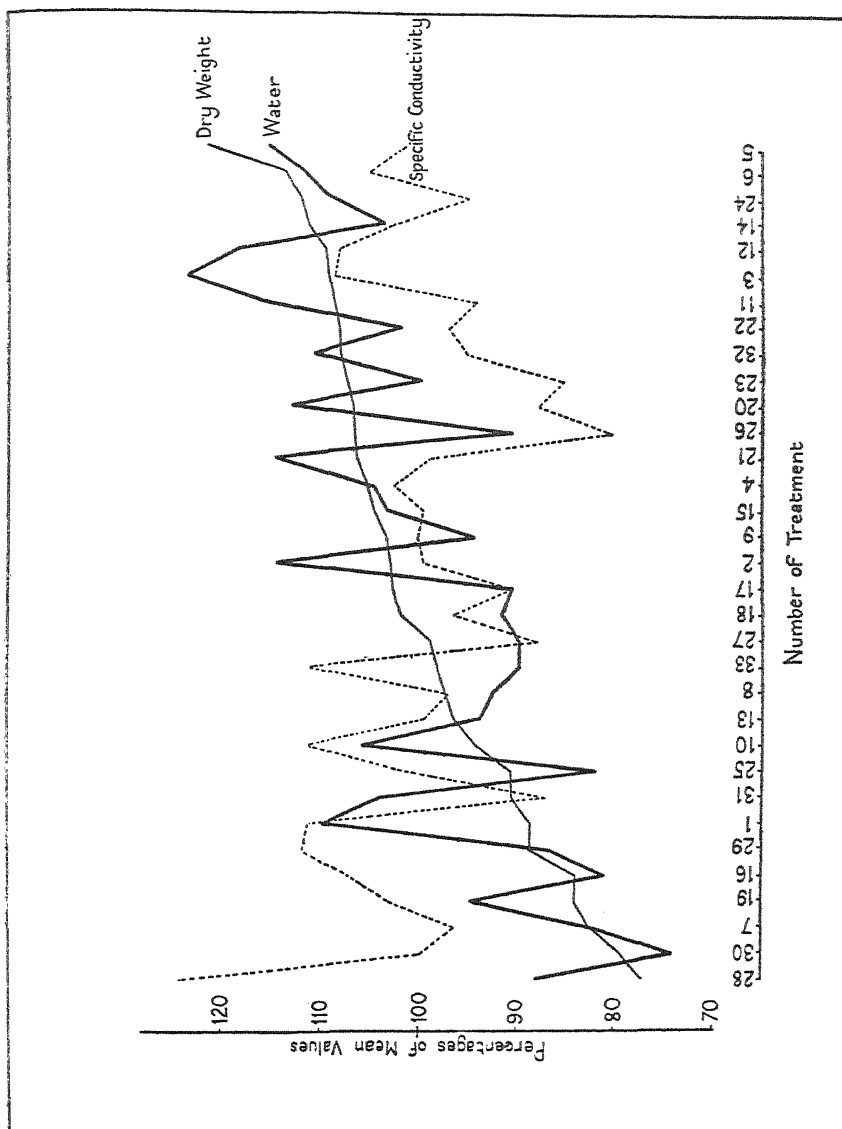
FIG. 1.   Values for dry weight, water, and specific conductivity of sap expressed as percentages of mean values (experiment 1).   Treatments are arranged in order of increasing dry weights.

weight = 9·4 per cent. and that for conductivity = 9·1 per cent.). If protein is used as the basis of expression instead of dry weight, the correlation coefficient between conductivity and water is increased from +0·48 to +0·57. The results are therefore in harmony with the salt hypothesis and suggest that the water content of the leaf is influenced both by the amount of anhydrous protoplasm (measured in terms of dry weight or protein) and by the salt concentration. In this experiment, foliar hydration was not apparently affected by differences in the size of the plants, for the correlation coefficient between the dry weight of the whole plant and water per 100 gm. dry weight in the leaf was insignificant ($r = -0·18$).

While the results of this experiment are in harmony with the salt hypothesis, it should not be lost sight of that the orthodox osmotic theory affords an equally good explanation, assuming either conductivity or depression of freezing-point of the sap to be a measure of osmotic pressure, for depression of freezing-point is also significantly correlated with water on either the dry weight ($r = +0·45$) or protein ($r = +0·50$) basis.

Richards and Shih (1940) have concluded from a study of the relationship between water content (water per 100 gm. dry weight) and the mineral composition of barley leaves that most of the observed variations in water content could be accounted for by the sodium and phosphate contents (expressed in terms of dry weight) with sodium playing a predominant part. Potassium showed little, if any, relation to succulence. Cotton differs from barley in that sodium is not absorbed to anything like the same extent as potassium or calcium (cf. Phillis and Mason, 1940). Analyses for the individual elements are not available for the present experiment, but estimates of total soluble salt are. Fig. 2 shows water and salt plotted against each other on both the dry-weight and protein basis. On the left the data for all 33 treatments are given, while on the right, where only treatments 1–27 (see Table I) are considered, the mean values for the high, medium, and low levels of potassium, sodium, and phosphorus (see Table I) are shown. It should not be forgotten that calcium varied inversely with sodium. In the upper part of the figure the values are expressed on the dry-weight basis and in the lower part on the protein basis. The correlation coefficients between salt and water amounted to +0·870 on the dry-weight basis and to +0·996 on the protein basis. The results do not indicate any specific mineral effect.

It may be that the difference between our conclusions and those of Richards and Shih lies in the fact that we have considered only elements in solution in the sap, whereas their results deal with total (i.e. soluble plus insoluble) amounts. Richards and Shih, moreover, did not take into consideration the 'size effect'.

V. Variation in Concentration (*Experiment 2*)

A. *Procedure.*

This experiment was designed to cover a wide range of concentration of nutrient solution. It consisted of two series differing only in nitrogen treat-
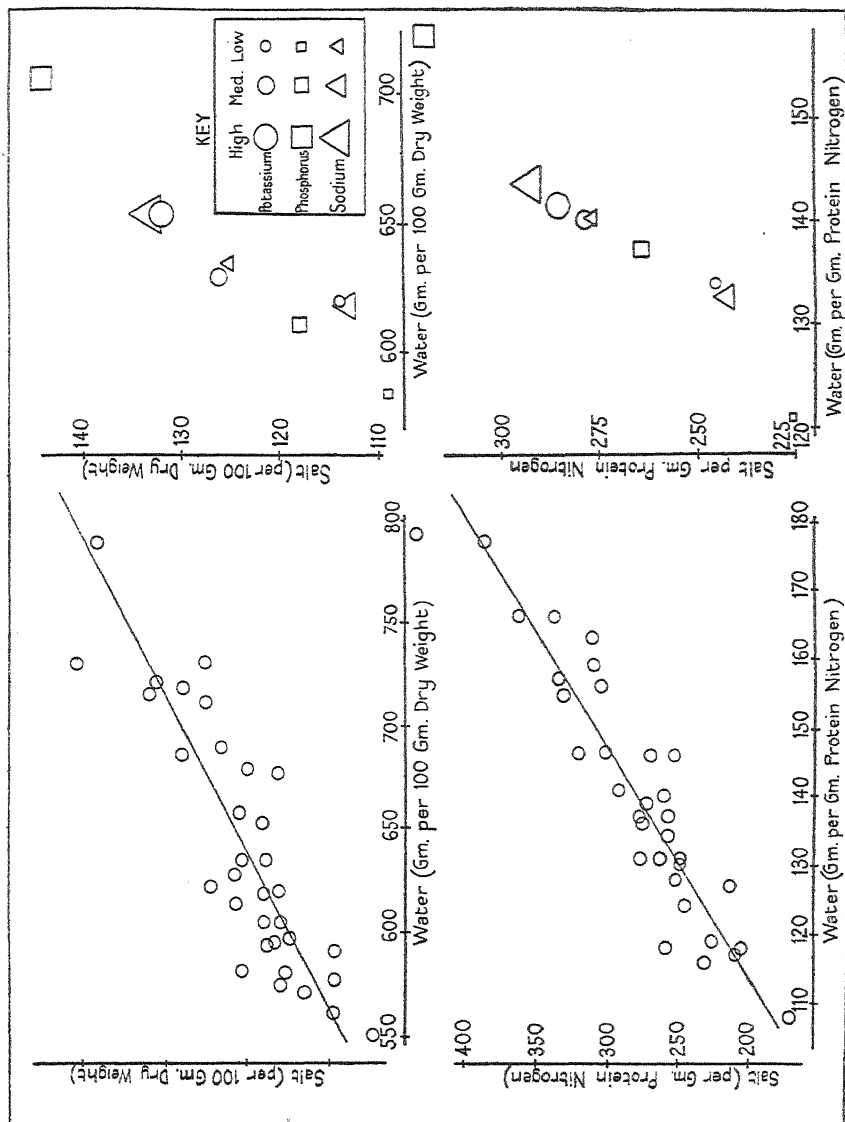
FIG. 2.   The *upper* two figures show salt and water content on the dry-weight basis plotted against each other for individual (33) treatment on the left, and for mean values for high, medium, and low levels of phosphorus, potassium, and sodium on the right.   The lower two figures show the same relationship calculated on the protein basis.

ment. The standard culture solution had the following composition: Nitrogen and potassium, 300 p.p.m. each; calcium and magnesium, 100 p.p.m. each; phosphorus, 50 p.p.m., and traces of iron and the minor elements. In the first series the concentration of nitrogen was kept constant at 300 p.p.m., while the concentration of the other nutrients varied from half to five times the standard
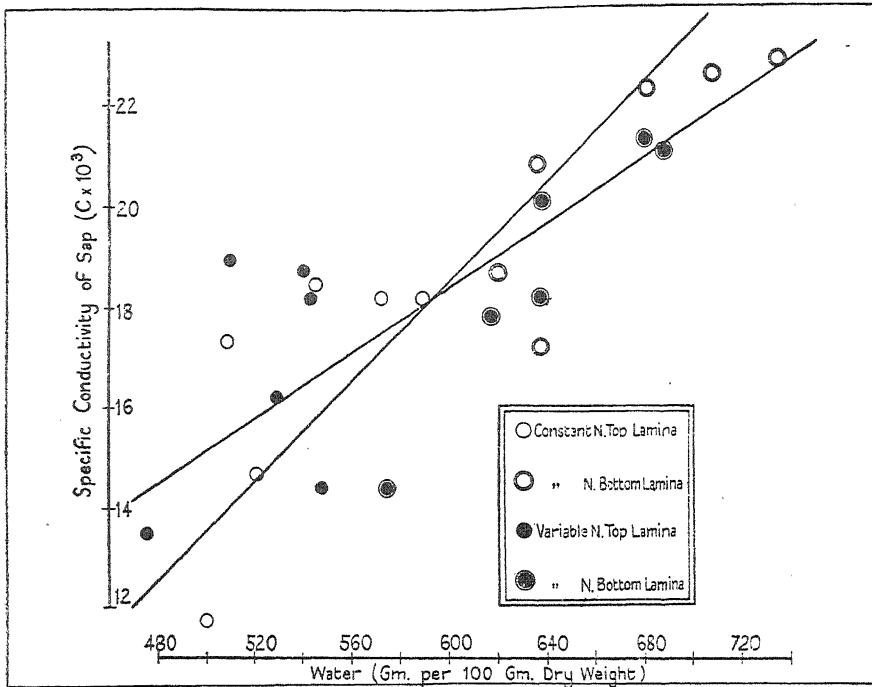
FIG. 3. Specific conductivity of sap plotted against water (per 100 gm. dry weight) for 'Top' and 'Bottom' leaves of *Constant* and *Variable* series (experiment 2).

concentration. This series will be referred to as the *Constant Series*. In the second series the concentrations of all the nutrients, including nitrogen, were varied over the same range. It will be referred to as the *Variable Series*.

The plants were grown in water culture in a greenhouse. The nutrient solution (2 litres per plant) was replaced weekly, while daily additions of water were made to replace transpiration losses. The greenhouse was kept damp and the plants frequently sprayed with water to avoid the 'size effect'. The plants were collected when 7 weeks old. The leaves were collected from two regions, those from the 4th to the 7th nodes from the apex formed the 'Top' leaves and those below the 7th node the 'Bottom' leaves.

B. *Results.*

In this experiment we proceed directly to consider the influence of sap conductivity on hydration. In Fig. 3 we show on the same graph the results for both ages of leaf and for both the Constant and Variable nutrient series.

Sap conductivity is plotted against water per 100 gm. dry weight. The correlation coefficient amounted to $+0.81$ and is, of course, fully significant. The regression lines are also shown on the graph. When hydration is considered on the protein basis the correlation coefficient amounts to $+0.78$, so that in this, as in the previous experiment, it is immaterial on which basis we consider hydration.

Inspection of Fig. 3 will make it clear that no distinction can be made between the two series, but that the two ages of leaf fall on different parts of the graph. The 'Bottom' leaves have, on the whole, higher conductivities and higher hydration values. This is also the case when the water is expressed in terms of protein. The correlation coefficients between hydration and conductivity for the 'Bottom' and 'Top' leaves of the two series are shown in Table II. It will be noticed that the coefficients for the 'Bottom' leaves are large and fully significant. For the 'Top' leaves the coefficients are not significant. For the Variable Series the coefficient is actually negative when water is expressed on the protein basis. To sum up, the results for the 'Bottom' leaves are in harmony with the salt hypothesis. This is also perhaps true for the 'Top' leaves of the Constant Series, but for the 'Top' leaves of the Variable Series other factors must be operating. Reference to Fig. 3 will, however, make it clear that the level of hydration in the 'Top' leaves fits into the same line as the 'Bottom' leaves.

TABLE II

Correlation Coefficients between Hydration and Conductivity for 'Top' and 'Bottom' Leaves of Constant and Variable Series

	'Bottom.'		'Top.'	
	Constant.	Variable.	Constant.	Variable.
Dry-weight basis . . .	$+0.83$	$+0.96$	$+0.72$	$+0.41$
Protein basis . . .	$+0.86$	$+0.90$	$+0.75$	-0.21

The correlation coefficients between hydration and the depression of freezing-point of the sap are shown in Table III. For comparison, the correlations between hydration and conductivity are also shown. The data for both ages of leaf and for both nutrient series have been used in calculating these correlations. It will be seen that the correlation between conductivity and hydration is fully significant and much higher than that between hydration and freezing-point depression on both the dry-weight and protein bases.

TABLE III

Correlation Coefficients for all Data between Hydration and Conductivity, Depression of Freezing-point and Depression of Freezing-point corrected for the Concentration of Total Sugars

	Dry-weight basis.	Protein basis.
Conductivity	$+0.81$	$+0.78$
Depression of freezing-point	$+0.38$	$+0.30$
Depression of freezing-point corrected for sugar	$+0.54$	$+0.43$

In the previous paragraph we pointed out that the sap conductivities were higher in the 'Bottom' than in the 'Top' leaves. The reverse is, as we showed some years ago (cf. Mason and Maskell, 1928), the case for sugars (i.e. total sugars). When the depression of freezing-point is corrected for the amount of sugar present, the correlation coefficients between hydration and depression of freezing-point are appreciably increased and become significant. This suggests that sugars play no part in determining hydration and that the concentration of electrolytes rather than the osmotic pressure of the sap is the factor affecting hydration.

VI. Variation in Nitrogen Supply (*Experiment 3*)

A. *Procedure.*

In experiment 1 nitrogen was the only element supplied at a constant level. Other elements varied over a wide range. In this experiment all other nutrients were kept at a constant level, while nitrogen supply varied widely. There were 8 levels of nitrogen supply, viz. 50, 100, 200, 500, 1,000, 1,500, 2,000, and 2,500 p.p.m. [The level of supply in experiment 1 was 400 p.p.m.] The levels of the other nutrients were as follows: potassium, 200 p.p.m.; calcium and magnesium, 100 p.p.m. each; and phosphorus, 50 p.p.m. The plants were collected when quite young (38 days old) to avoid the 'size effect'.

B. *Results.*

In this experiment, as in the two previous ones, the bulk of protoplasm was the chief factor in determining the weight of water in the leaf, but whereas in the first two experiments removal of the bulk factor by expressing water in terms of dry weight made possible strong positive correlations between water per 100 gm. dry weight and conductivity, the opposite happens in this experiment. The correlation coefficient between water per 100 gm. dry weight and conductivity in this experiment was negative ($r = -0.89$) and is fully significant. Either dry weight is here an unsatisfactory indicator of bulk, or conductivity is not affecting hydration as in experiments 1 and 2, or some new factor is intruding itself and obscuring the conductivity/water relation. The correlation between conductivity and water expressed on the protein basis is, however, high and fully significant ($r = +0.86$). This might suggest that protein is a more satisfactory basis than dry weight on which to express hydration. As we shall have occasion to consider this matter further in the discussion, we will postpone further comment.

VII. Discussion

The three experiments have thus far been considered separately. The correlation coefficients between sap conductivity and hydration are summarized in Table IV. The results for hydration are shown both for the dry-weight and the protein bases. The correlation coefficients using the combined data for the three experiments are also shown.
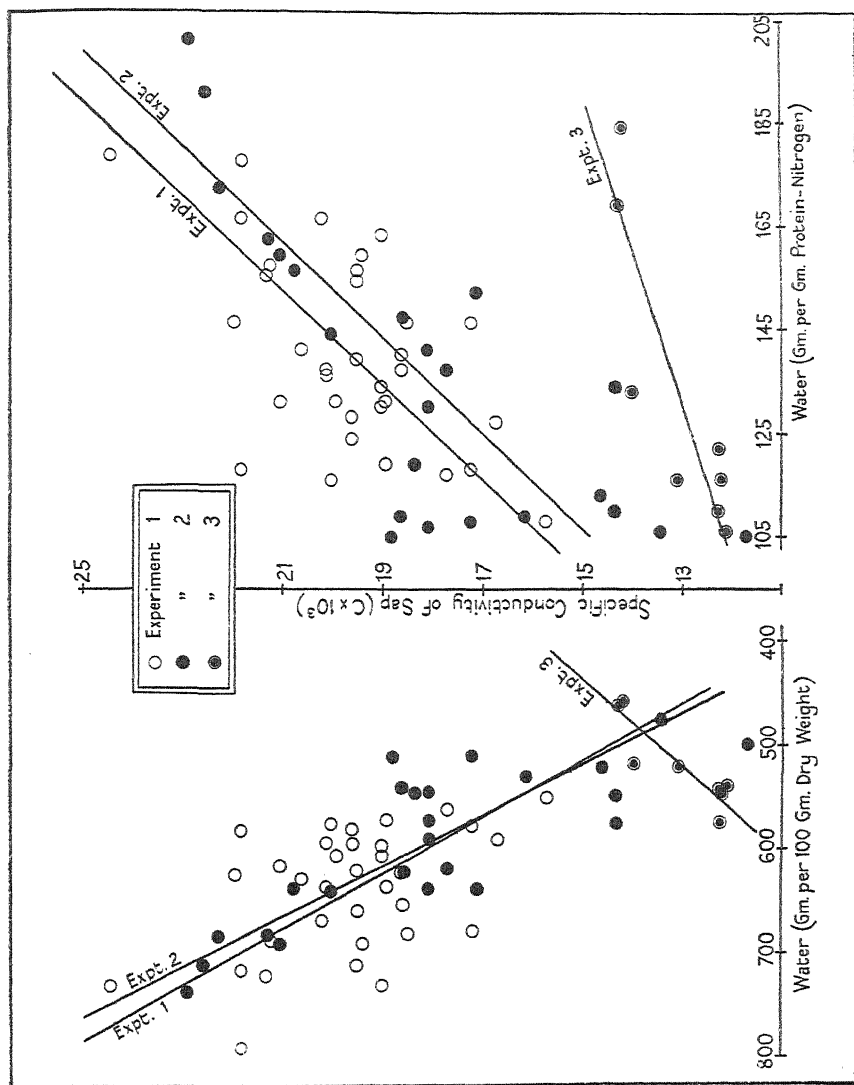
FIG. 4. Specific conductivity of sap plotted against water per 100 gm. dry weight on the left, and against water per gm. protein-N on the right, for all experiments. The mean regression lines for each experiment are also shown.

On the dry-weight basis, the correlations are positive for experiments 1 and 2, but negative for experiment 3. For the three experiments the correlation is positive and surprisingly high. All the correlations are, of course, significant. On the left of Fig. 4 we have plotted sap conductivity against water per 100 gm. dry weight for the three experiments. The means of the regressions of each experiment are also shown. It will be seen that these means for experiments 1 and 2 nearly coincide, but that the mean line for experiment 3 lies across the other two. The general level of the results for experiment 3 lies, however, on the regression lines of experiments 1 and 2. It would thus appear that sap conductivity may have been an important factor in determining hydration in experiment 3. The negative correlation suggests that in addition to conductivity another factor has been operating in this experiment.

The correlation coefficients between sap conductivity and water per gm. protein-N (see Table IV) are all positive and significant. The correlation using the combined data of the three experiments is also positive, but not as high as that on the dry-weight basis. The results are shown graphically in the right half of Fig. 4. The mean of the regressions of each experiment is also shown. It will be seen that these lines for experiments 1 and 2 are parallel and not far from one another, while the mean regression line for experiment 3 lies quite apart.

It seems clear that the results for experiment 3 on both the dry weight and the protein bases are anomalous. Nor is this surprising when the complexity of the salt-hydration relation and the difficulty of finding a basis for hydration are remembered. We are, moreover, still quite in the dark as to the part played by metabolism, pH, &c. Then the resistance of the wall and tissue tensions must play some part.

TABLE IV

Correlation Coefficients between Specific Conductivity and Water on the Dry-weight and Protein Bases

	Dry-weight basis.	Protein basis.	r for P = 0·05.
Experiment 1 . .	+0·475	+0·571	0·349
„ 2 . .	+0·809	+0·777	0·400
„ 3 . .	−0·888	+0·862	0·707
All experiments . .	+0·730	+0·570	0·240

Nor is it yet known how nitrogen supply affects the volume relations of cytoplasm and vacuole. For these reasons it seems to us remarkable, in experiments covering as wide a range of nutrient supply as the three experiments reported, that the correlations between sap conductivity and hydration should be so strong.

VIII. Summary

1. Three experiments are described. The object of these experiments was to ascertain to what extent changes in the composition and concentration of the nutrient supply affected foliar hydration and how such changes in hydration were brought about. Previous results have led us to suggest that the weight of

water in the leaf is determined by two factors. The first is the *weight* of anhydrous protoplasm (the Bulk Factor), and the second is the *hydration capacity* of protoplasm. We have also postulated that the hydration capacity of protoplasm is controlled by its salt content.

2. In the first experiment the nutrient varied both in composition and in concentration. Water in the leaf was significantly correlated with both dry weight and protein, which we have used as indicators of anhydrous protoplasm. When this 'Bulk Factor' was removed by expressing water in terms of dry weight and/or protein, hydration was significantly and positively correlated with sap conductivity. The results are fully in accord with the salt hypothesis of foliar hydration. No evidence of a specific effect of any single element on hydration was detected.

3. In the second experiment the concentration of the nutrient solution was varied. Again hydration and sap conductivity were strongly correlated. Old leaves were found to have higher conductivities and higher hydration than young leaves. It was also observed that conductivity was more strongly correlated with hydration than was freezing-point depression of the expressed sap. The correlation between freezing-point depression and hydration was appreciably increased when the freezing-point depression was corrected for the concentration of sugar in the sap.

4. In the third experiment there was a wide range of nitrogen supply. Sap conductivity and hydration were negatively correlated on the dry-weight basis and positively correlated on the protein basis. The causes of this difference are discussed.

5. When the data for the three experiments are combined the correlation between hydration in terms of dry weight and sap conductivity is positive and surprisingly high. Over the wide range of nutrient supply covered by these three experiments, it would seem that hydration is chiefly determined by the level of salt concentration.

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Experimental and Analytical Studies of Pteridophytes

I. Preliminary Observations on the Development of Buds on the Rhizome of the Ostrich Fern (Matteuccia struthiopteris Tod.)

BY

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With Plate V and twenty-eight Figures in the Text

I. INTRODUCTION

RHIZOMES of the ostrich fern (*Matteuccia struthiopteris* Tod.) are apparently quite devoid of buds, though the formation of buds which subsequently develop into rhizomes is characteristic of the erect shoot. If, however, a typical rhizome has its apical meristem removed or destroyed and is maintained under suitable cultural conditions, buds may develop at points along its entire length. These buds grow directly into erect plants bearing juvenile and later normal foliage leaves.

Although there have been many investigations of the occurrence and development of buds in ferns, the observations made by the writer on the ostrich fern have apparently not hitherto been recorded.[1] The data which have accrued from this and related investigations should, in the writer's view, contribute eventually to a fuller understanding of various aspects of plant development and organization as exemplified by the Ferns.

II. EXTERNAL AND INTERNAL MORPHOLOGY OF PLANTLINGS

If pieces of decapitated rhizome are placed in peat in a cool greenhouse or in an incubator at 22–25°C., small buds, soon having the appearance of diminutive fern plants and referred to here as 'plantlings', make their appearance in the course of 3 to 5 weeks; some typical specimens are illustrated in Text-figs. 1–8. The buds arise singly or in linear groups in the shallow grooves characteristic of the rhizome of this fern. Young buds appear as rounded outgrowths of turgid, pale-green tissue; later the plantling has the appearance of a short shoot with an apical region and bearing a lateral, fleshy, non-laminated, slightly curved, first leaf. Considerable numbers of roots soon begin to emerge and additional leaves develop at the apex of the shoot. The first two fleshy leaves may be approximately opposite. Later, circinately

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[1] It seems probable that similar observations have at one time or another been made by those interested in fern culture; the writer, however, has found no reference to the subject in the morphological literature available to him.

curved primordia become apparent; on unrolling, these are seen to be pin-
nately lobed leaves.
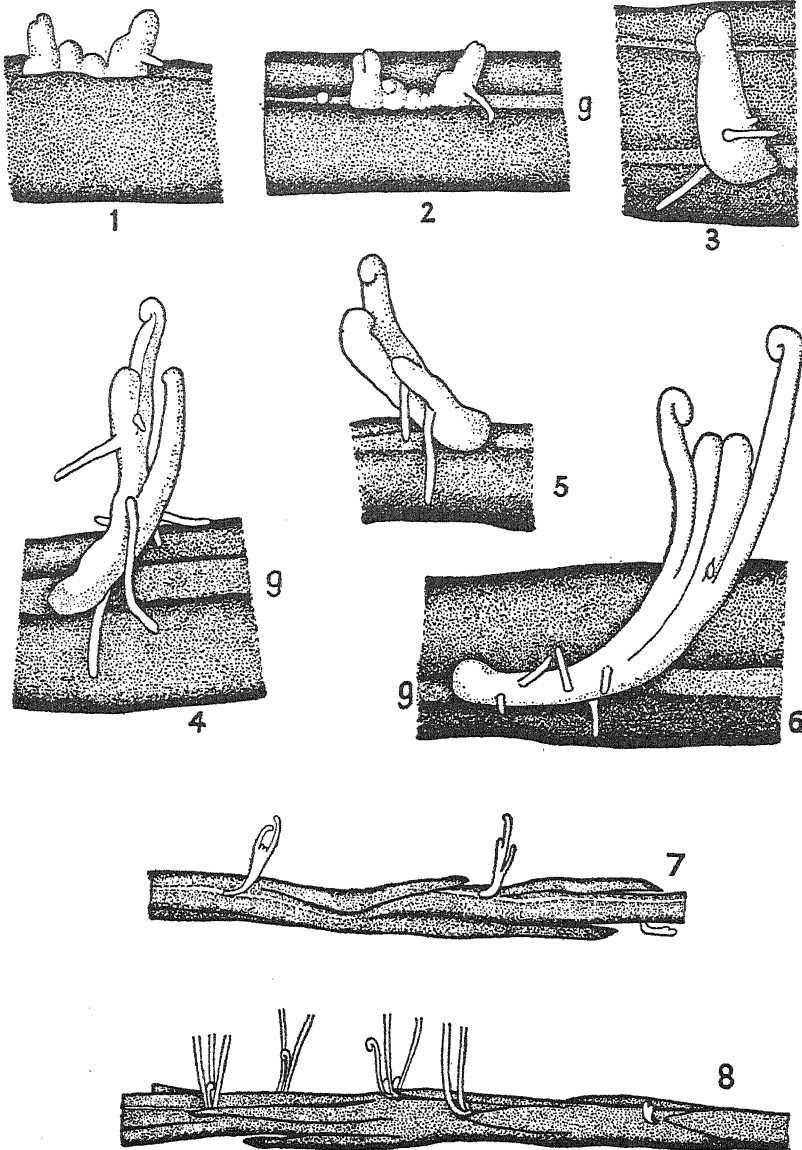
TEXT-FIGS. 1–8. Buds and plantlings developing on decapitated rhizomes of
    Matteuccia struthiopteris.   g, groove. (Figs. 1–6 × 3·5, Figs. 7 and 8 × 0·75.)

The relation of plantlings to the parent rhizome is illustrated in Text-
figs. 7 and 8; they are seen to occupy a position which, from external in-
spection, may be defined as either (*a*) in the longitudinal axis of a scale-leaf,

but at some distance away from the leaf axil, or (*b*) in a lateral position in relation to a leaf-base. Both axillary buds and lateral, leaf-base buds have been described in different ferns (Bower, 1923). It will be an integral part of the present study to consider the positional relationships of plantlings.

Rhizomes grown in a temperate glasshouse have mostly yielded single buds and single plantlings at each meristematic region, whereas those in the incubator have tended to produce groups of plantlings. On further growth during the summer typical small erect plants were formed, and towards the end of the growing season, in the temperate house, one or more horizontal stolons began to emerge from the basal region of the bulky terminal bud.

The experimental evidence on the causal relationships between decapitation and plantling development is still insufficient for a full or definitive statement, but it may be noted that control pieces of rhizome which had not been decapitated showed slight elongation in the apical region and did not produce plantlings or only did so at a late stage when apical activity appeared to have become quiescent, whereas decapitated rhizomes and fragments of rhizomes invariably produced plantlings abundantly.

Observations have been made on serial sections which illustrate in terms of internal morphology the successive stages in the development of plantlings. As the writer intends to use these data in a later paper, where a consideration of the factors operative in the organization and development of plantlings will be attempted, only a brief reference to the internal structure will be made here.

At the base of the shoot the vascular system consists of a medullated protostele, the xylem consisting of a ring of tracheides surrounding a central pith. This configuration is not modified by the insertion of the two traces of the first-formed leaf. Higher up, below the insertion of the second leaf, the stele opens out into a gutter-shaped structure, an internal endodermis being present. The two traces of the second leaf are conjoined with the free edges of this structure. In older plantlings, a typical dictyostelic condition is established, this being associated with a phyllotaxis of approximately one-third. In the upper region of stout erect shoots the primordia of what will become laterally outgrowing stolons or rhizomes can be observed. At the apex differentiation proceeds from a well-defined two-sided apical cell; leaf primordia develop from single, large, meristematic cells situated on the flanks of the apical cone. These observations are in close agreement with published descriptions of the apex of the adult shoot (Mekel, 1933).

A detailed comparison of rhizome plantlings with prothallial plants, i.e. young sporophytes (Campbell, 1886), suggests itself as affording interesting materials for analytical studies of development and organization.

III. Origin of Rhizome Buds

Induced buds always arise in particular positions. By means of serial sections, data of the type illustrated in Pl. V, Fig. 6, have been consistently

obtained. The place of origin of the plantling-bud is at the surface of the rhizome in the region of conjunction of two meristeles, in other words, at the
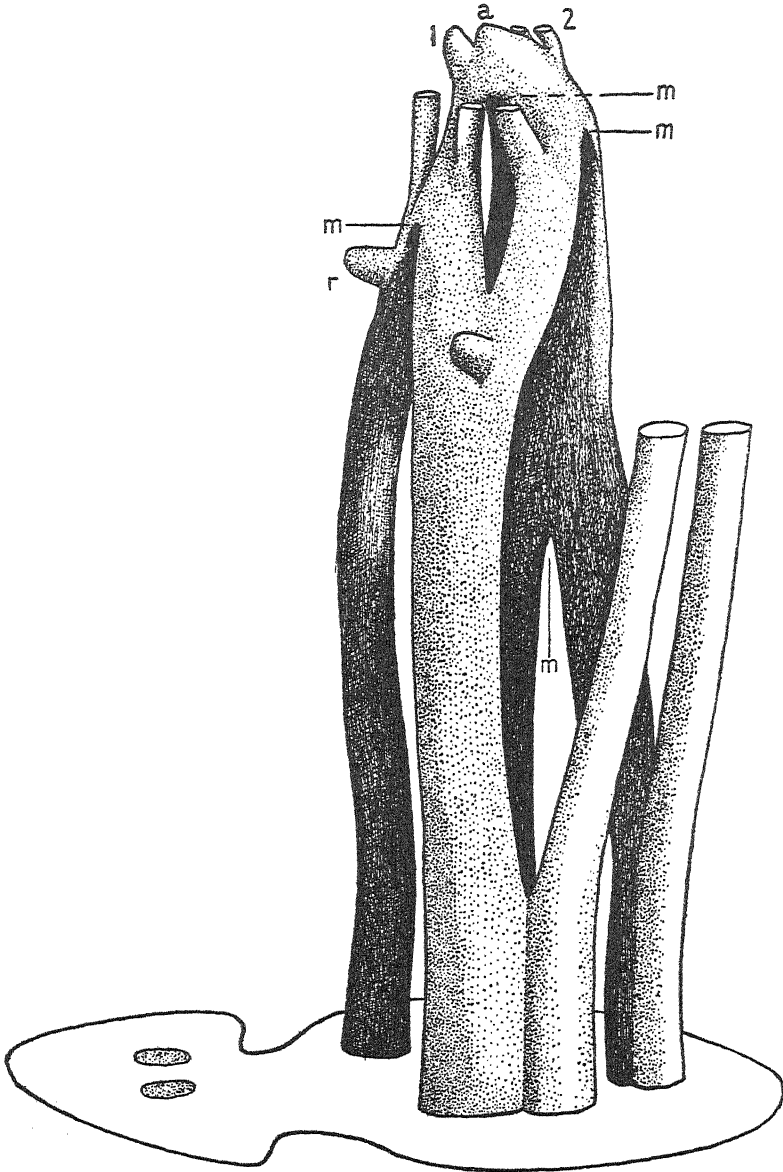
TEXT-FIG. 9. Reconstruction of the vascular system of the terminal region of a rhizome (×22). Detached meristems occur opposite the four points of meristele conjunction (*m*). These are the points of origin of induced plantlings. *a*, vascular system below terminal apex; 1, 2, primordia of two leaves; *r*, root.

upper extremity of a foliar gap in the stelar meshwork. The reconstructed vascular system, Text-fig. 9, shows four meristele unions (*m*), opposite each

of which the development of a plantling could be induced by removal of the apical meristem.

Three possibilities relevant to the development of plantlings suggest themselves: (i) that small inconspicuous buds have in fact been organized but are not evident on external inspection; (ii) that buds are of adventitious origin,
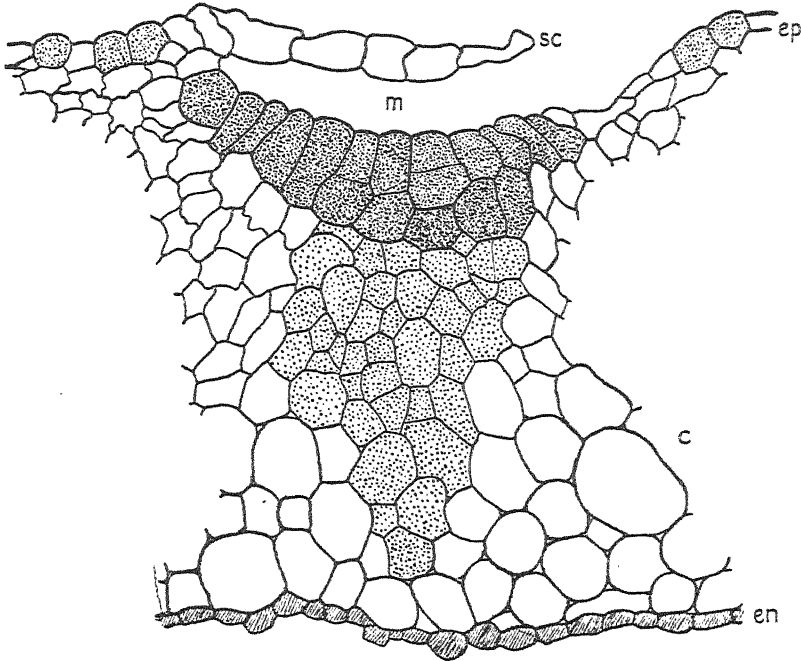
Text-fig. 10. T.S. rhizome (semi-diagrammatic), showing meristematic cells (heavily stippled) in groove, at commencement of plantling development; cells showing similar staining reaction below (lightly stippled). (× 180.) *m*, detached meristem; *sc*, scale; *en*, endodermis; *ep*, epidermis; *c*, cortex.

their development being referable to growth and division of normal epidermal and sub-epidermal cells; (iii) that some morphological or anatomical feature other than (i) or (ii) above is involved. Investigations have shown that (i) and (ii) may be ruled out as having no part in the origin and development of plantling-buds.

Sections cut transversely through a rhizome at the point of junction of two meristeles show that in the groove where a plantling-bud may subsequently originate, a characteristic layer of superficial, rectangular cells is consistently present, Text-fig. 10 and Pl. V, Fig. 2. These cells are considerably larger than the epidermal cells; they possess dense protoplasmic contents, and give the staining reactions and have the distinctive appearance of meristematic cells. In tangential section the meristematic cells are seen to occupy an area of ellipsoidal to lenticular shape situated in the rhizome groove. They are quite distinct from the elongated, thick-walled epidermal cells, Text-fig. 11, but

being concolorous with them, tend to escape observation when viewed under low magnifications in reflected light. For the most part they occur in rows, are of rectangular outline, and have apparently been formed by the transverse division of cells elongating in the direction of the long axis of the rhizome, Pl. V, Fig. 4. In radial longitudinal section the meristematic cells appear approximately as in transverse section, Pl. V, Fig. 5. They are thus rectangular
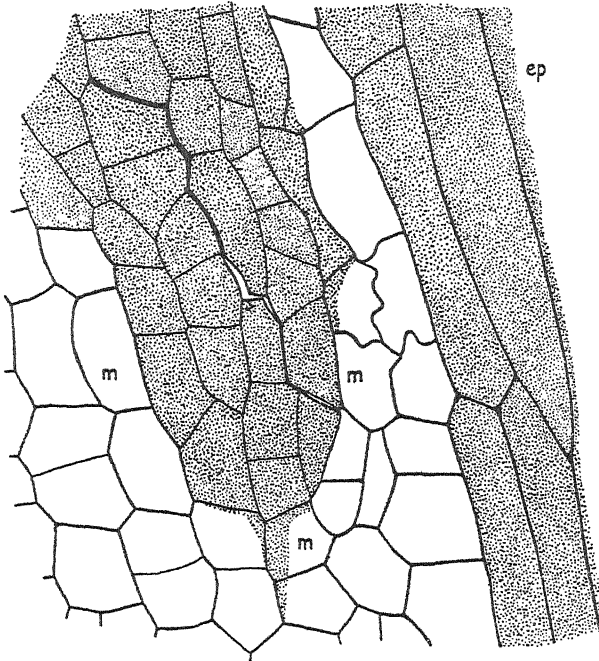
TEXT-FIG. 11. Surface (tangential) view of the tissue of a detached meristem; on the right are typical elongated epidermal cells (*ep*). (×340.) As a result of growth and cell-division in the meristematic cells (*m*), the yellowish cuticle is flaking off.

prisms, measuring 35–50, 27–30, and 40–50 μ in the longitudinal, tangential, and radial directions. On their tangential margins the meristematic cells are bounded sharply by the elongated epidermal cells; at their upper and lower limits they become enlarged and elongated and merge with the epidermal cells, the region of transition being characterized by the outgrowth of scales which overarch the meristematic region.

In the quiescent condition of an adult rhizome the meristematic cells possess a yellowish-brown cuticular covering like that of normal epidermal cells. They tend to resist the absorption of stains and in transmitted light, even after staining, have brownish-yellow contents and dark-brown walls; they then show a considerable contrast with adjacent epidermal and cortical tissues. With the beginning of growth, as in experimentally treated adult rhizomes, the meristematic cells expand and bulge outwards, the cuticle is ruptured and flakes off, and the cells lose their yellowish colour and readily

absorb stains. Cell-divisions follow which may be transverse and periclinal, but other division patterns may also appear. The whole or a considerable part of the meristematic region may enter on a phase of active growth or several centres of growth distributed without any apparent order over the meristematic region may become active more or less simultaneously; the former condition results in the development of a single strong bud, whereas
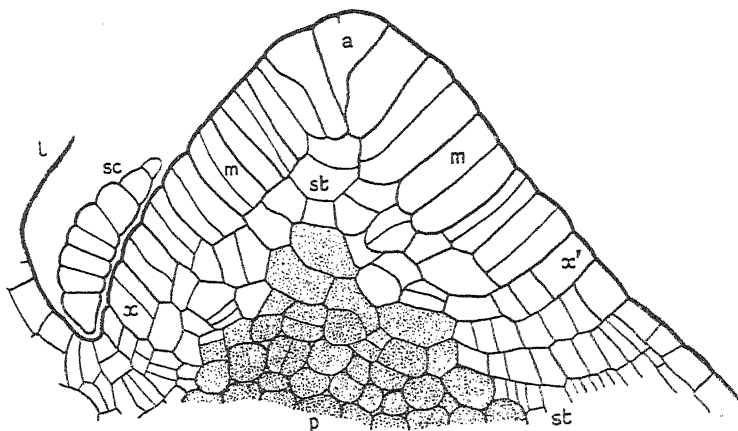
TEXT-FIG. 12. L.S. rhizome apex, showing apical cell (*a*), and meristematic cells (*m*) of the apical meristem extending down the flanks of the cone to *x* and *x'*. (×255.) *st*, differentiating stelar tissue; *p*, pith; *sc*, scale; *l*, leaf.

the latter yields several buds. That the vascular system of the plantling is not joined to that of the rhizome is shown in Pl. V, Fig. 6.

The origin of these meristematic regions now requires consideration. They are, in fact, traceable back to the apical meristem of the rhizome.

IV. THE APICAL MERISTEM

Text-fig. 12 and Pl. V, Fig. 1, show median longitudinal sections through a rhizome apex. On both sides of the apical cell (*a*), which is of the so-called two-sided type (Mekel, 1933), the superficial layer consists of conspicuous, radially elongated rectangular cells, these being direct products of segments derived from the apical cell. Internally, periclinal walls separate off an inner mass of approximately equi-dimensional cells which constitute the stele in its earliest stage. The aforementioned rectangular cells are of distinctive appearance, possess dense protoplasmic contents like the apical cell, and by virtue of their origin and their approximately terminal, superficial position may be expected to share in a high degree the physiological properties of the apical cell. Staining tests support this view, the reactions of the apical cell and of the flanking rectangular cells being similar, but quite distinct from those of the adjacent differentiating stelar tissue within and of the developing epidermal and cortical tissues below. These conspicuous rectangular cells will now be referred to as *meristematic cells*. Text-fig. 12 shows that meristematic cells extend from the point denoted by *x* on the left flank of the meristem to *x'* on the right.
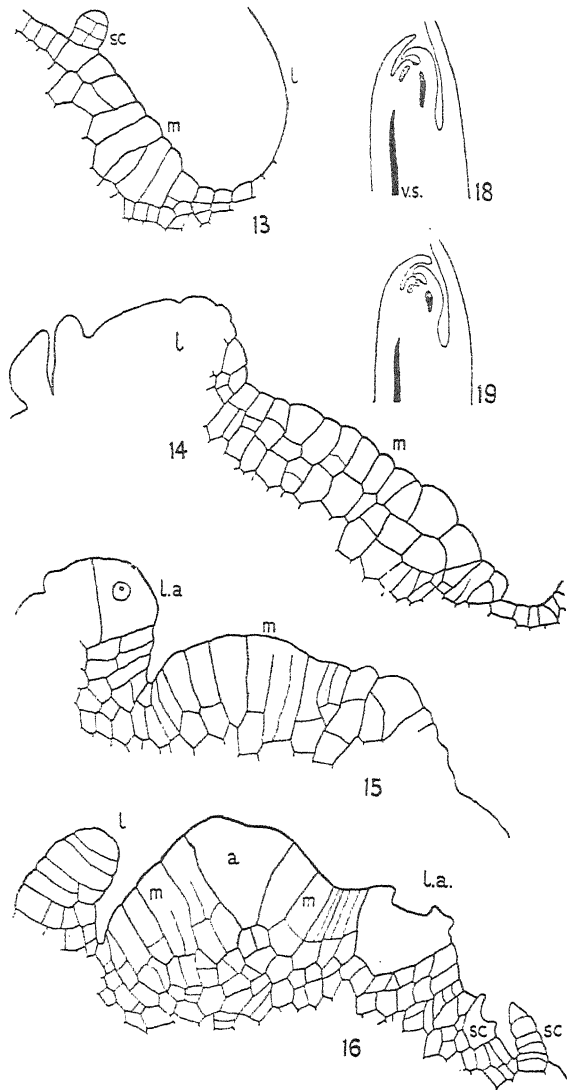
TEXT-FIGS. 13–21. Serial longitudinal sections through a rhizome apex. Figs. 13–17 show the distinctive meristematic cells (*m*), which constitute the apical meristem, extending well down the sides of the apical cone. Figs. 16 and 20 are in the median plane; Figs. 13, 14, 15, 18, and 19 illustrate the position and appearance of the meristematic cells on one flank of the apex, and Figs. 17 and 21 the corresponding data for the flank diametrically opposite;. *v.s*, vascular strand; *l.a*, leaf apex; *a*, apical cell; *l*, leaf; *sc*, scale. (Figs. 13–17, ✕ 128, Figs. 18–21, ✕4.) (*For* TEXT-FIGS. 17, 20, and 21, *see opposite.*)

Below *x* meristematic cells have been differentiated into the smaller units which constitute the superficial tissues of the leaf axil; below *x'* meristematic cells have undergone a series of rapid divisions by periclinal and anticlinal walls and epidermal and cortical tissues will in due course be differentiated. From some of the recently divided meristematic cells in the transitional regions below *x* and *x'*, scales have developed.

Text-figs. 13–21 give further information of the distribution of meristematic cells in the terminal region; they show their positional relationship to
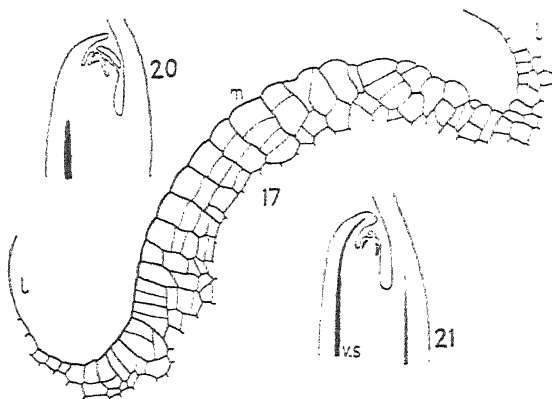
TEXT-FIGS. 17, 20, and 21.

developing leaf primordia and also to what extent they can be traced down the sides of the apical cone. In transverse sections through the apical cone it can further be observed that meristematic cells are no longer in evidence where the rhizome surface is tenanted by a developing leaf primordium or by the much distended base of an older leaf, or in those regions of the shoot where rapid radial and tangential expansion is taking place. During these developments the original, distinctive character of the meristematic cells disappears. In certain positions, however, small groups of them still persist, e.g. those shown diagrammatically opposite leaves 4 and 5 of Text-fig. 23 and Pl. V, Fig 7.

Text-fig. 22, reconstructed from serial transverse sections, indicates the distribution of meristematic cells in the terminal region of the rhizome. This distinctive layer, which includes the apical cell, constitutes the actual *apical meristem*[1] as distinct from the epidermal, cortical, and stelar tissues in their earliest stage of development. As indicated in Text-fig. 22 the *apical meristem* extends to a young leaf primordium, leaf 1 on the left, and also downwards as ribbons of diminishing width towards the axils of leaves 2, 3, and 4; but it disappears immediately in front, that is, in a region where very considerable growth enlargements, associated with the development of the bases of leaves 2 and 3 and of the shoot, have taken place. Lower down, in approxi-

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[1] The term *meristem* is often used without being defined or the limits of its fundamental tissue being indicated. In the present instance, as also in leptosporangiate ferns in general, the apical meristem can be precisely indicated, and it is in this sense that the writer now uses the term.

mately the median plane of leaf 5 (shown here in transverse section), but well above its junction with the rhizome, a small, superficial, circular to ellipsoidal area (*m*) consisting of recognizable meristematic cells, has persisted. This area,
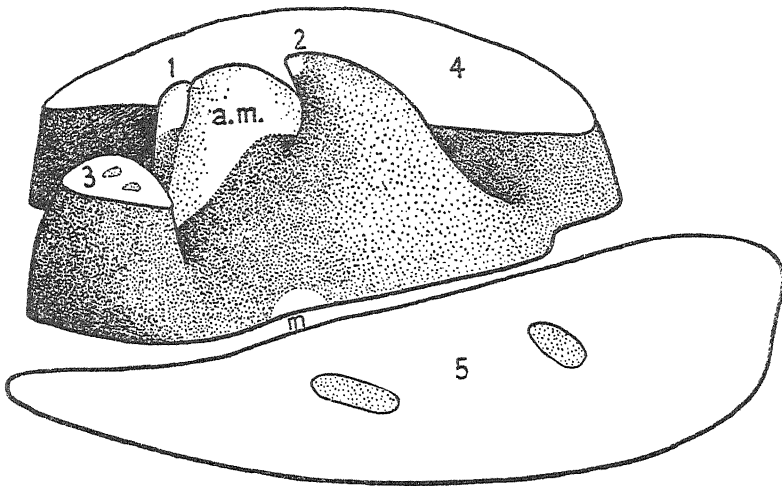
TEXT-FIG. 22. Reconstruction, from serial transverse sections of a rhizome apex; *a.m*, apical meristem; *m*, detached meristem; 3, 4, 5, leaves cut transversely; 1, 2, primordia of most recently formed leaves. (×40.)

originally part of that region of the apical meristem which extended down towards the axil of leaf 5, has become isolated during the several growth enlargements already considered; the other meristematic cells, which at an earlier stage surrounded this residual group, have contributed to this growth development and have undergone extensive division and differentiation into epidermal and cortical tissue. As apical growth continues other similar meristematic areas will become isolated and will be found in certain positions along the length of the rhizome; it is from them that the plantlings described in earlier sections originate.

These isolated meristematic areas may for convenience be described as constituting *detached meristems*, i.e. they are detached portions of the *apical meristem* as specified above. From the evidence available they should not be described as buds, since (1) although they possess meristematic potentiality they show no definite bud organization, and (2) they may give rise, under different conditions, to one bud or to several buds, or merely to a coralloid mass of parenchymatous tissue, an indication that they are indeterminate in respect of their further development. Here it is appropriate to mention that buds have on occasion been obtained from the underlying tissue when the superficial layer of a detached meristem has been removed.

V. THE FURTHER DEVELOPMENT AND PERSISTENCE OF DETACHED MERISTEMS

The cells of the apical meristem immediately overlie and are in direct contact with the plerome; the latter is, in fact, derived from the meristematic

cells by periclinal cleavages. Detached meristems situated near the apex may likewise abut directly on the developing stelar tissue (Pl. V, Fig. 7), or may be separated from it by one or two newly formed layers of developing cortical parenchyma derived from the meristematic cells. Lower down, as the rhizome attains its adult size the detached meristems undergo some tangential enlargement and the under-lying cortical tissue increases as a result of further tangential divisions of the meristematic cells (Pl. V, Figs. 8 and 9). As this cortical development is of limited extent the detached meristem eventually occupies a slightly sunken position. These developments can also be followed, though with less ease, by means of longitudinal sections (Text-figs. 24–8, Pl. V, Fig. 5); a con-siderable elongation of the detached meristem takes place during the de-velopment of the shoot to the adult condition.

TEXT-FIG. 23. Transverse section through the base of the apical cone of a rhizome, show-ing the position of eight leaves ($l1$, $l2$, &c.) and of two detached meristems (m) opposite leaves 4 and 5 ; st, recently differentiated stelar tissue; ep, epidermis; $v.s$, vascular strands of leaves. ($\times 57$.)

As apical growth proceeds, a ma-jority of the meristematic cells on the margin of the apical meristem become successively transformed into epi-dermis and cortical parenchyma. From a consideration of the facts of shoot-development from the apex backwards it is apparent that the tangential growth and enlargement of the cortical and epidermal tissues must be maxi-mal opposite the widest part of the foliar gaps in the dictyostelic vascular system, and minimal at the points of conjunction of shoot meristeles; it is only in proximity to the latter positions that detached meristems are found. Whether these are positions of minimal mechanical stress or minimal nutri-tion in relation to supplies diffusing out from the vascular system, or whether other factors are involved, such as the localized distribution of growth-inhibiting substances, must at present remain uncertain. It has been seen that in respect of bud-formation these detached meristems remain inactive until the dominating influence of the rhizome-apex is removed.

VI. THE ORIGIN AND DEVELOPMENT OF RHIZOMES

Experimentally-induced plantlings have no vascular connexion with the shoot stele, Pl. V, Fig. 6. In this respect they differ from the normal stolon- or rhizome-buds which appear as outgrowths from the erect shoot, Pl. V, Fig. 3. These stolon-buds, however, resemble plantlings in respect of their origin, i.e. they also develop from detached meristems. The inhibition of further development which is a normal feature in the detached meristems of

rhizomes is not an equally constant feature in those of the erect shoot. In the latter there are, apparently, periods of inhibition and other periods when bud-development takes place. In the writer's view, the mode of development of a bud on the erect shoot does not provide evidence of a side-tracked
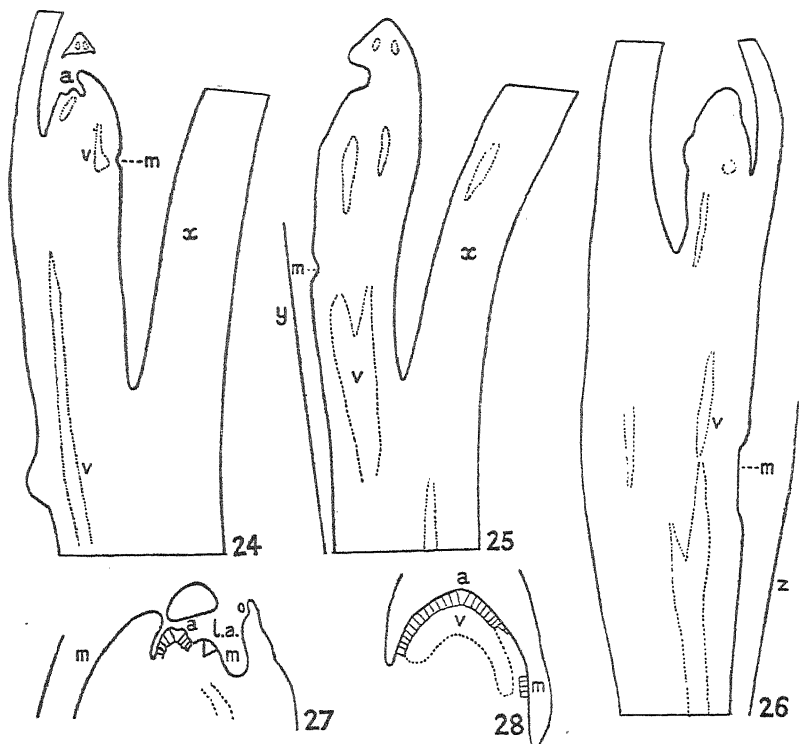
TEXT-FIGS. 24–8. Longitudinal sections of rhizomes showing the positions of detached meristems (*m*) in relation to the associated leaves, *x, y, z*; *a*, rhizome apex; *v*, vascular tissue; outline indicated by broken lines; *l.a*, leaf apex: Figs. 24 and 25 from one rhizome. Fig. 26 from a second rhizome. Figs. 27, 28, diagrams of apical region showing relative positions of first two detached meristems (*m*); *a*, apical cell. (Figs. 24, 25, 26, × 11; Figs. 27 and 28, × 36.)

dichotomy or morphological event of that category; the bud develops from a detached meristem some distance below the apical meristem (about the fifth youngest leaf, approximately, in the writer's preparations), the early state of differentiation of the shoot-stele admitting of the conjunction with it of the vascular strand proceeding from the developing bud.

Mekel (1933) after summarizing the views regarding the origin of stolons or rhizomes in the ostrich fern, i.e. that they are adventitious in origin (Hofmeister and Velonovsky), or that they are due to normal branching of the main axis (Mettenius and Luerssen), comes to the conclusion that the latter view is correct, and states that in relation to every leaf there is to be found a stolon or rudimentary primordium thereof. This finding is confirmed by the writer's observations, but a more fundamental conception of

what constitutes 'normal branching' is obviously required. Data of the type submitted here contribute towards this end.

VII. DISCUSSION

Bower (1923) has stated that many ferns show dichotomy of the apex, that buds may be produced in an axillary position, or that they may arise in extra-axillary positions related to the leaf-base. He also notes that in the second and third categories above there may be a question of 'what relation, if any, the buds so produced bear to the apex of the main shoot; that is, how far they may be held to be of the nature of distal branchings of it, diverted into a lateral position and delayed in their origin'. Further, he points out that while it may be possible to interpret the buds about the leaf-base in ferns in terms of distal branching unequally developed, there are cases which are obviously not of that nature and that each requires separate solution. The normal erect-shoot-buds and induced rhizome-buds of the ostrich fern appear to provide examples of such cases. They are not referable to distal branching unequally developed nor are they formed adventitiously, but they do stand in a definite relation to the shoot apex in that they originate from groups of meristematic cells which at one time constituted part of the apical meristem.

These observations (i) demonstrate a unity of origin of superficial meristematic tissue throughout the plant, and (ii) suggest that a solution to comparable problems in other ferns may be sought along similar lines.

VIII. SUMMARY

1. The horizontal rhizome of the ostrich fern (*Matteuccia struthiopteris*) is devoid of buds, but they can be induced along its entire length by the removal of the apical meristem. Such buds develop into erect plantlings, and eventually into normal adult plants.

2. Anatomical studies show that induced plantlings always arise superficially on the rhizome in proximity to points of conjunction of two meristeles of the dictyostelic vascular system. At these points small areas of distinctive meristematic cells are present in a quiescent condition in the normal rhizome.

3. It is shown that these potentially formative regions, described as *detached meristems*, are referable in origin to the *apical meristem;* the latter can be specified as a superficial layer of distinctive meristematic cells directly derived from the apical cell and clothing the flanks of the apical cone. In relation to the character of the growth development, parts of the apical meristem become detached and persist in definite positions. The horizontal rhizomes, which develop laterally on the erect shoot, also originate from detached meristems.

4. The data obtained have been considered in relation to those for bud-formation in the ferns generally. In the ostrich fern it is concluded that rhizome-buds and induced plantlings owe their origin neither to distal apical branching unequally developed nor to adventitious development, but

they do bear a definite relation to the shoot apex in that they develop from groups of meristematic cells which at one time constituted part of the apical meristem.

It is a pleasure to acknowledge the assistance received from Mr. E. Ashby in microscope preparations and photographic illustrations.

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DESCRIPTION OF FIGURES IN PLATE V

Illustrating Professor C. W. Wardlaw's paper on *Matteuccia struthiopteris* Tod.

(All figures are from untouched photographs)

Fig. 1. L.S. of rhizome apex, showing the apical cell, superficial, rectangular, meristematic cells covering the sides of the apical cone, and within, the developing vascular tissue and central pith. (× 225.)

Fig. 2. T.S. of an adult rhizome, showing the distinctive meristematic cells of a slightly sunken detached meristem. (× 225.) These may be compared in size and shape with those of the apical meristem shown in Fig. 1 above.

Fig. 3. T.S. of erect shoot, showing a rhizome- or stolon-primordium and its relation to two conjoined rhizome meristeles. (× 100.)

Fig. 4. Surface view of a detached meristem in the quiescent condition, from a tangential hand-section of an adult rhizome. (× 55.)

Fig. 5. Longitudinal radial section of a rhizome showing a slightly sunken detached meristem situated just below the terminal apical cone. A narrow zone of cortex separates the detached meristem from a meristele which bifurcates in the downward direction at this point. (× 180.) This illustration was made from the same section as Text-fig. 24.

Fig. 6. Transverse section of a rhizome with attached plantling, the latter being cut somewhat transversely through its basal region and longitudinally through its region of attachment. The plantling has no vascular connexion with the rhizome stele, but it will be seen that its place of origin is in proximity to a region of meristele conjunction. (× 35.)

Fig. 7. T.S. of rhizome near the apex showing two detached meristems, i.e. below and top left; in each a row of four distinctive meristematic cells can be seen; on the left, a leaf-base; within, developing vascular tissue and central pith. (× 150.)

Figs. 8 and 9. Illustrate two detached meristems, progressively farther down the rhizome than those shown in Fig. 7. The position of the meristems in relation to adjacent rhizome meristeles and the formation of cortical parenchyma by tangential divisions of the meristematic cells are clearly shown. (× 180.)
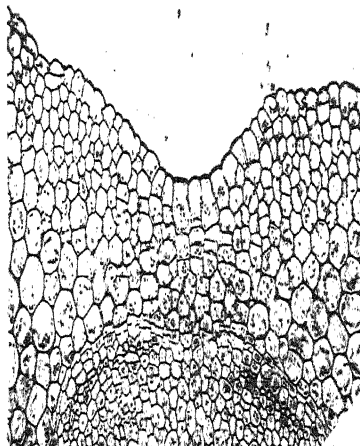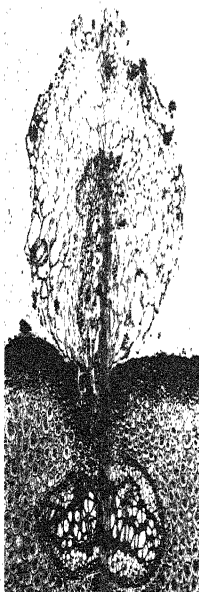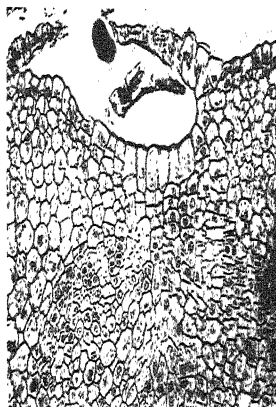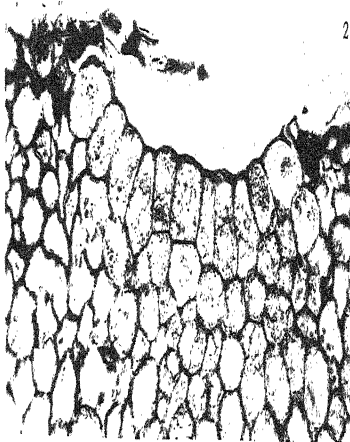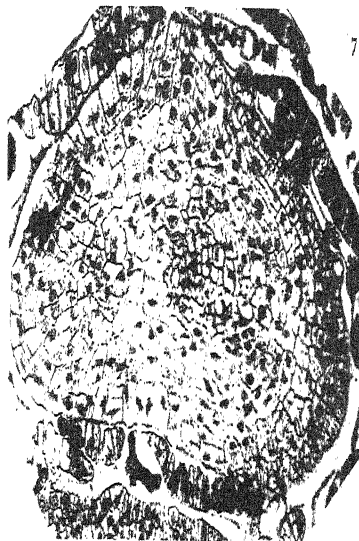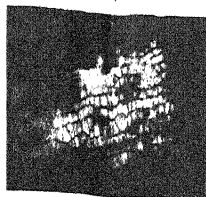
The Morphology and Anatomy of Fegatella conica in relation to the Mechanism of Absorption and Conduction of Water

BY

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With four Figures in the Text

FEGATELLA (*Concephalus*) *conica* is one of the largest and most common members of the Marchantiaceae. Its habitat varies from situations such as the sides of streams, and damp hedge-banks, to the bases of very damp walls. It grows in large, dark-green tufts with broad dichotomously branched thalli. As a result of this tufted habit there is a tendency to a marked overlapping of the thalli, while the extending tips become directed obliquely upwards.

Bowen (1935) showed that the overlapping ventral scales of *Fimbriaria bleumeana* aided in the conduction of water, in the form of capillary films, from the base of the thallus to the inclined tips. Experiments were performed to investigate whether Bowen's findings also applied to *Fegatella conica*.

The scales of the species under investigation run in two overlapping rows on the ventral surface of the thallus. They are small, reddish-brown, oval to kidney-shaped appendages attached to the sides of the 'midrib' or thicker portion of the thallus at their basal ends, whilst their free apical regions point towards the centre of the thallus, i.e. away from the 'lamina' or wing portion of the thallus. Thus the 'midrib', which projects slightly ventrally, is closely covered, on either side, by these two rows of scales, enclosing a 'median furrow' richly supplied with tuberculate rhizoids. These rhizoids pass back in rigid tufts and many pass down between the scales into the soil. The smooth-walled rhizoids, on the other hand, arise in dense masses immediately outside the scales.

Entry of water and solutes.

The experimental methods employed to determine the source of water supply were identical with those already described by the author in a similar investigation on *Pellia epiphylla* (Clee, 1939), i.e. the use of solutions of 'vital' stains to determine water transport, with the result that, within five minutes, the stains had reached the tips of all the plants under investigation and had stained the scales and rhizoids with their respective coloration. It was therefore assumed that the scales and rhizoids were effective in conducting capillary films of liquid from the bases of the thalli to the inclined tips. A point of interest noted was that the stain travelled far more rapidly along the 'median furrow'

enclosed by the ventral scales and harbouring the tuberculate rhizoids, than by means of the tufted smooth-walled rhizoids situated outside the rows of scales. Large drops of stain were also detected between the scales which closely overlapped and protected the apical growing-point. This method of conduction obviously provided the growing tips with a ready supply of water; so much so, that hand sections of plants so treated showed that the apical cells had absorbed the various coloured solutions before the cells of the ventral surface were stained at all. Accordingly one important function of the ventral scales is apparently that of supplying the growing-point with a constant flow of liquid in the form of capillary films. So rapid is this flow that in a number of experiments it was found that the stain always reached the tip of a portion of a thallus up to 6·0 cm. long within less than 5 minutes from the time that its base was placed in the solution.

In an attempt to determine the actual point of entry of external liquids portions of thallus, previously washed and dried of surplus moisture by blotting-paper, were supported in Petri dishes, with their cut surfaces just under the surface of 0·5 per cent. solutions of selected 'vital' stains. At varying intervals the plants were removed and sections were cut at different points and examined microscopically to detect the stained areas and so to determine the possible point of entry of the stains. It was found that the most successful stains were Congo red, neutral red, methylene blue, and diamin black, the last-named being dissolved in a 1 per cent. solution of sodium chloride. Other plants were similarly placed in 0·1 per cent. solutions of potassium nitrate, and still others in a similar concentration of ferric chloride, the sections being respectively mounted in diphenylamine dissolved in concentrated sulphuric acid and in ammonium sulphide solution.

It was observed that the smooth-walled rhizoids absorbed the stains far more rapidly than the tuberculate rhizoids. Even at the end of one hour, when all the smooth-walled rhizoids were well coloured by the stain, no traces of colour were observable in the majority of the tuberculate rhizoids. Furthermore, the only thallus tissue which was coloured was that confined to the lateral portions of the 'midrib' and the innermost parts of the 'lamina', that is, the regions of origin of, and those adjacent to, the smooth-walled rhizoids.

Examination of sections taken at intervals showed that from the bases of the smooth-walled rhizoids on either side of the 'midrib' the stain spread both downwards into the ventral tissue of the thallus and also inwards, following the distribution of the mucilage canals, as indicated by the arrows in Fig. 1; finally it spread from this 'midrib' region out into the wings.

Although the stain passed down from its original point of entry in the region of the smooth-walled rhizoids into the ventral tissue of the 'midrib', the small-celled, compact tissue, found immediately above the 'median furrow', where the tuberculate rhizoids arise, was invariably the last to show the presence of stain, in spite of the fact that capillary films of the various coloured liquids were easily detected between the scales and the tuberculate rhizoids, and entry in this region might be expected. However, sections treated with

0·5 per cent. of colluidin blue and thionine respectively revealed no trace of mucilage in this area of small-celled tissue; while those cells directly concerned with absorption of stain were rich in mucilaginous contents, showing a similar reaction to that given by the very large mucilage ducts which they surround, and which traverse the whole length of the 'midrib' in a longitudinal direction. Certain cells in the 'lamina' or wing portions of the thallus
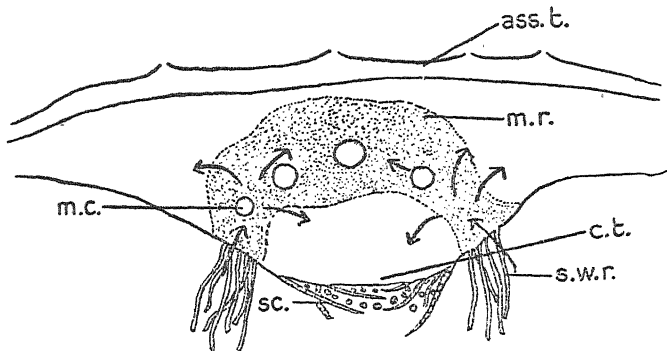
Fig. 1. Plan of a transverse section of thallus of *Fegatella conica*. The arrows denote the path of entry of stains while the shaded area denotes the distribution of the so-called 'mycorrhiza': *ass.t.* assimilating tissue; *mc.* mucilage cell; *sc.* scale; *m.r.* mycorrhizal region; *c.t.* compact. tissue; *s.w.r.* smooth-walled rhizoid. (× 30.)

also gave a positive reaction for mucilage. The tips and basal portions of the smooth-walled rhizoids also showed the presence of mucilage, but the tuberculate rhizoids always failed to give the reaction.

It is clear from Fig. 1 that the point of entry and the path of spread of the various stains closely followed the so-called 'mycorrhizal' region which extends, as seen in transverse section, in a kind of semicircular arch through the 'midrib' region, the bases of the arch terminating in the region of the smooth-walled rhizoids. The hyphae can be seen within these rhizoids and probably enter the thallus by this route, especially since two 'invaded' regions, one on either side of the thallus in the vicinity of the attachment of the smooth-walled rhizoids, can first be seen, these two regions then extending until they meet to form the arch referred to above. Some, at least, of the invaded cells give a reaction for mucilage. The small-celled compact tissue at the ventral surface of the 'midrib', on the other hand, was free from both mucilaginous contents and fungal hyphae. Golenkin (1902) suggested that the function of the 'mycorrhiza' of the Marchantiaceae was that of water storage and drought resistance, but Cavers (1904) held that this function was scarcely to be attributed to the tissue of an hygrophilous form such as *Fegatella conica*. The author's observations suggest that the position of the fungal hyphae in the thallus—a position corresponding with the point of entry and path of spread of water—is that most suitable to the growth and well-being of the endophyte.

From the 'midrib' the stain appeared to spread towards the under surface of the 'lamina', and then dorsally to the assimilating tissue, last of all reaching

the very small cell of the compact tissue in the proximity of the region of attachment of the tuberculate rhizoids.

Microscopical investigation of the cells adjacent to the smooth-walled rhizoids and in the region traversed by the liquids showed the presence of pits on their walls. Cavers (1904) described these as large starch-containing cells with walls thickened by anastomosing 'fibres', the unthickened portions re-
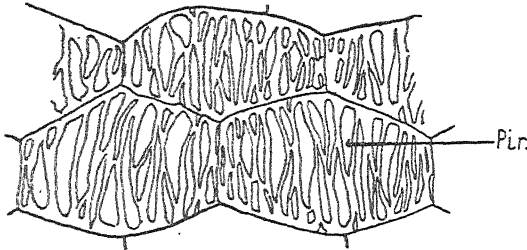
FIG. 2. Cells in the absorptive regions of the thallus showing the irregular arrangement of the pits. (× 480.)

maining as slit-like pits. The writer, however, found that these cells were thickened by anastomosing bands of cellulose, the whole forming irregular slit-like pits as shown in Fig. 2. The walls of these pitted cells stained blue with Delafield's haemotoxylin and with 5 per cent. methylene blue, denoting the presence of cellulose. Further tests giving a positive reaction for cellulose were the blue coloration of the walls on the application of Schultze's solution and again the slow dissolution of the cell walls in Schweitzer's reagent.

The distribution of these pits on the walls of the cells most concerned with the transport of water suggests that they play some part in such transport from the smooth-walled rhizoids to the large mucilaginous ducts in the central region of the thallus, and thence into the lateral and dorsal portions of the thallus.

Since the mucilage ducts function as reservoirs of water it might be expected that, so long as protoplasmic permeability permits the passage of dissolved substances, the mucilage will show a greater concentration of the solutes absorbed than will the surrounding cells. This was found to be the case, and tests for the presence of solutes gave some indication of the rate of movement within the ducts; this, however, was found to be very slow, suggesting that the movement within the mucilage ducts corresponded only to that which might be expected from the rate of utilization of water by the surrounding cells; that is to say, the rate of movement within the mucilage ducts suggests that these are not routes of rapid transport but merely reservoirs for local supply.

The function of the rhizoids.

The functions of the rhizoids of the Marchantiales have always been debatable. Leitgeb (1881) stated that both types of rhizoid attached the plant to the

soil and absorbed water, but that the tuberculate rhizoids also strengthened the thallus. Kamerling (1897), however, was of the opinion that the turgor of the cells of the ventral tissue of the thallus was sufficient to maintain the plant in a rigid condition, and proved this by removing the rhizoids, when the rigidity of the thallus remained unaltered. Kny (1890) believed that the tubercles served the purpose of preventing the rhizoid walls from collapsing from lateral pressure or lack of water. Haberlandt (1896) wrote that the tubercles, by increasing the area of cytoplasm increased the area of absorption. Like Cavers (1904) the writer found that neither the smooth-walled nor the tuberculate rhizoids collapsed to any extent when the plants were kept under dry conditions. Furthermore, with the exception of very young rhizoids and the tips of the older rhizoids, it was found that the smooth-walled rhizoids had no protoplasmic contents. From his own observations the writer suggests that the smooth-walled rhizoids have largely an absorptive function, while the tuberculate ones act as organs of anchorage and at the same time facilitate external conduction of liquids. The smooth-walled type of rhizoid absorbs stains far more quickly than the tuberculate ones, and the chief route of entry of water and solutes into the thallus lies immediately internal to them   The tuberculate rhizoids are far more rigid, and either run back parallel with the thallus within the 'rhizoidal groove' or else grow down into the substratum as very long straight strands. These latter clearly function in anchorage while those rhizoids growing parallel with the undersurface of the plant and surrounded by the envelope of protective scales provide an excellent means of increasing the surface available for the conduction of external films of water right up to the growing tip and thus to the developing sex organs and sporophyte. The tubercles, or ingrowths of the wall, in these rhizoids conduce to their rigidity, and therefore help to maintain them in parallel formation and so aid the external supply of water.

The sex organs.

The structure of the sexual receptacles of *Fegatella conica* is well known, and the application of similar methods to those already applied by the author to *Pellia epiphylla* (1939) and by Bowen (1935) to *Fimbriaria bleumeana* showed that these receptacles, and the sex organs which they bear, are supplied with water in exactly the same way as in the other two cases. The supply to the antheridial receptacle, and thence to the antheridia, is facilitated by the sinking of the receptacle in a groove in the vegetative tissue, this groove extending completely round the circumference of the receptacle except for the small anterior region in the neighbourhood of the apical cells. Here the encirclement of the receptacle is completed by the upgrowth of the ventral scales, and thus the antheridial cushion is surrounded by a continuous 'moat' of environmental liquid.

The conditions under which sperms are liberated have given rise to some diversity of view. Cavers (1904) maintained that the antheridia swell when in

contact with water and thus come into contact with, and press against the paraphyses, in the cavities. The resulting pressure squeezes out the sperms on warm sunny days. Further movement of the sperms under these conditions was not explained. Goebel on the other hand stated that raindrops are essential for the liberation of the motile sperms, and that these raindrops splash the archegonial receptacle and so form a continuous film for the movement of the sperms. The writer's observations make it clear that, under
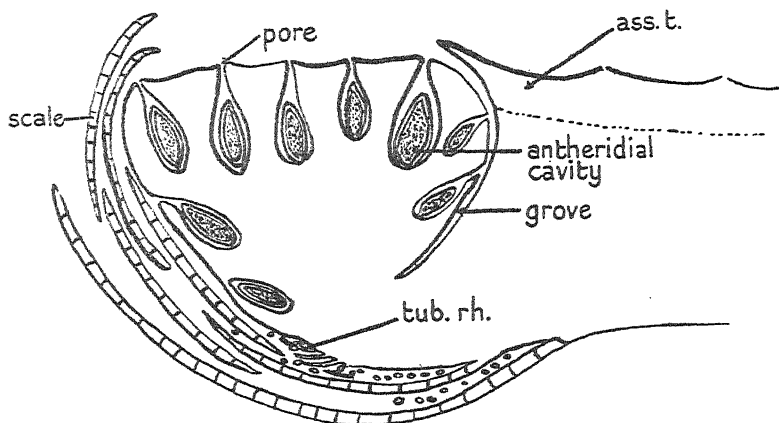
FIG. 3. Diagrammatic representation of longitudinal section of the antheridial receptacle of *Fegatella conica* showing the overlapping arrangement of the scales, and the groove; *ass.t.*, assimilating tissue, *tub.rh.*, tuberculate rhizoid.

normal environmental conditions, a supply of water is maintained over the surface of the sexual receptacles adequate to ensure both the liberation of the male gametes and a medium for their migration.

The young carpocephalum is situated in a groove-like depression (Fig. 4), continuous except for the anterior region where it is completed by upturning scales, the whole having the effect of encircling the structure with liquid, as in the case of the antheridiophore.

Stains accumulating in this groove rapidly travelled up the rhizoidal groove of the carpocephalum stalk (a continuation of the 'median furrow' of the thallus), this upward passage being facilitated by the additional surface provided by the upward-pointing tuberculate rhizoids.

From the stalk of the carpocephalum, just as in the case of *Fimbriaria bleumeana* and *Pellia epiphylla*, the stains travelled along the archegonial canals and stained the oospheres long before the cells of the venter had absorbed the stain. Within 30 minutes of placing of the base of the thallus in the stain, examination of more mature carpocephala showed that the widened neck cells and oospheres or oospores were deeply stained. The most spectacular results were obtained with 'vital' red and neutral red, and with both of these stains the canals of the archegonial necks appeared microscopically as red lines between the colourless neck cells, whilst the

oospores appeared as dark red spots surrounded by a colourless envelope of venter cells.

The sporophyte.

The fact that the developing sporophytes were readily supplied, as in the
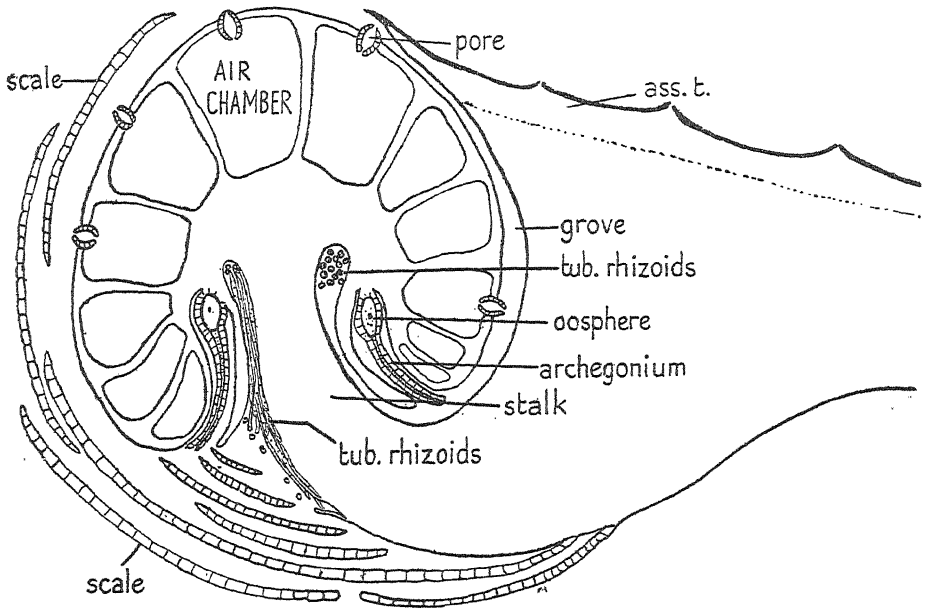
FIG. 4. Diagrammatic longitudinal section of a young carpocephalum showing the overlapping ventral scales and the groove caused by the upgrowing thallus, the whole enclosing the stalk. ($\times$ 35.)

previous cases reported, with films of liquid which travelled down the very widely opened and withering necks of the archegonia and between the calyptra and the segmenting sporophyte was confirmed by numerous observations. Transverse sections of the carpocephala showed distinct films of stain all round their outer surfaces, round the stalks, round the inner surface of the heads of the carpocephala, and between the sporophytes and the surrounding calyptra.

A marked elongation of the carpocephalum stalk followed the differentiation of the sporophyte into foot, seta, and capsule, but not until a few days prior to the shedding of the spores did these stalks fully elongate. Thus during the greater part of the time required for its development the sporophyte is surrounded by the tissues of an almost sessile carpocephalum with an abundant water supply.

When thalli with such elongating stalks were placed with their bases in various stains, these rapidly reached the head of the carpocephalum and

within a few minutes could be detected between the now widely opened calyptra and the capsule wall of the sporogonia. Some films of stain penetrated even more deeply, reaching the region of the foot and seta and even staining the foot long before the gametophytic tissue of the carpocephalum showed any coloration.

Very soon a distinct coloration of the spores and capsule walls of the sporogonia could be observed, even before the seta showed any such coloration. This is, to some extent, explained by the reaction for mucilage given by the capsule wall and the intersporal material within the capsule. These regions might therefore be expected to absorb water directly from without and with the water the dissolved dye, the coloration resulting from the latter indicating the path of entry. It would seem, therefore, that the capsule absorbs its supplies of water direct and not from the foot via the seta.

As the seta elongated at maturity, bringing the capsule well below the level of the head of the carpocephalum for effective spore dispersal, little evidence could be obtained for internal conduction of liquid from the foot to the capsule, though, in a few cases, slight colorations were detected in the central elongated cells of the seta. Coloration was, however, very evident in the outer cells of the seta, suggesting that these, like those of the capsule, had absorbed the stain directly from the exterior. It seems evident, therefore, that the seta plays little, if any part in transport of water to the developing and maturing capsule.

As in *Pellia epiphylla* (Clee, 1939) microscopic examination showed that there is no organic connexion between the foot of the sporophyte and the gametophytic tissue of the carpocephalum. In fact, so loosely embedded are the sporophytes in the tissue of the carpocephalum that they can easily and cleanly be removed intact by gently pressing the head of the carpocephalum between the fingers. These observations tend to support the views previously expressed by the writer (1939) and by Bold (1938) and Isaac (1941) that the sporophytes of the Hepaticae are to a considerable extent independent of the gametophytes for nutrition, depending mainly on the gametophyte for support and protection.

SUMMARY

In *Fegatella conica* water travels in the form of capillary films between the tuberculate rhizoids and ventral scales to the growing tip where it is readily absorbed.

The smooth-walled rhizoids have largely an absorptive function, whilst the more rigid tuberculate rhizoids aid in the external conduction of liquids and in anchoring the thallus to the substratum.

The entry of liquids into the internal tissues is aided by the presence of numerous mucilage cells and, more importantly, by the presence of large cells with irregularly pitted walls situated immediately internal to the regions of origin of the smooth-walled rhizoids.

The sex organs receive practically the whole of their water supply from external sources.

The water is retained in the narrow groove between the thallus and the antheridial receptacle, and also in the narrow spaces left between the antheridia and paraphyses in the antheridial cavities.

The oospheres, segmenting oospores, and sporogonia are largely supplied with water from the exterior; this passes up from a basin-like depression surrounding the base of the carpocephalum and is aided in its upward passage by the upward-pointing tuberculate rhizoids in the rhizoidal groove of the stalk of the carpocephalum. It travels down the necks of the archegonia, reaching the oospheres, and later the developing sporophyte. The segmenting embryo, the surrounding calyptra, and the enveloping head of the carpocephalum provide adequate surfaces for the retention of water films for this purpose.

The developing sporophyte with its foot, seta, and capsule is capable of absorbing water all over its external surface from the surrounding capillary films, and the seta appears to play little part in conduction from the foot to the capsule.

The sporophyte is largely independent of the gametophyte for its water supplies and food material.

The writer wishes to extend his thanks to Professor F. A. Mockeridge for her valuable advice and criticism during the progress of this work.

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Observations on the Spiral Structure of Somatic Chromosomes in Osmunda with the Aid of Ultraviolet Light

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With Plates VI and VII and two Figures in the Text

INTRODUCTION

THIS paper may be regarded as a direct continuation of work already published by one of us (Manton, 1939), though it breaks new ground both in methods and in material. The observations principally concern chromosome structure at metaphase of the second division in the germinating spore of the fern *Osmunda regalis* L. Discussion will be confined to a few cells only and to almost a single stage, since the data obtainable by the new methods might otherwise become too voluminous to handle. The use of the ultraviolet microscope, in particular, has made it possible for us to observe, with precision and on a large scale, many details which could only be deciphered with difficulty, if at all, by visual light. The technical advance which this

statement expresses is perhaps as important as the observations themselves as a motive for attempting publication at this time.

The study of spiral structure in somatic as opposed to meiotic chromosomes has hitherto been a matter of extreme difficulty. The usual methods for revealing the structure cannot be applied through any considerable thickness of cells or cell membranes, a fact which excludes the use of most of the meristematic tissues in plants in which mitoses are principally to be found. One observation has indeed recently been made on a cell from a root apex of Fritillaria after prolonged exposure to cold (Darlington and La Cour, 1942), but most of the very limited number of other records, as far as these can be traced in war-time, refer to special types of cells such as pollen grains,[1] pollen tubes,[2] and the tapetum.[3] Moreover, even when the spiral structure is successfully exposed, the observations required often lie so near to the limit of resolution of the microscope that details may be obscured from this cause. This was undoubtedly the case in *Osmunda*, and in consequence it was necessary in 1939 to accept a single imperfect count of the number of coils in one tapetal chromosome as the only direct evidence available regarding the spirals in the somatic chromosomes of this plant.

The experimental work which the problem has required has consisted on the one hand in the devising of new methods for the treatment and mounting of the material so as to expose the spirals to view, and on the other in establishing a procedure for photographing the required structures in ultraviolet light. The ultraviolet microscope, as is well known, is capable of greater powers of resolution than the ordinary microscope owing to the shorter wave-length of the light employed. A number of difficulties had, however, to be overcome before it could be applied to this material. The necessity of presenting the object on quartz instead of glass met with the initial difficulty that the quartz cover-slips proved too brittle to withstand the type of handling required for mounting the spores in the first instance. The sensitiveness of the apparatus to the presence of stains was a further obstacle. Most cytological stains either fluoresce or are opaque to ultraviolet light, and yet some preliminary staining is indispensable to the cytologist, for suitable cells are at best infrequent and cannot be selected unless the chromosomes can be clearly seen.

The general principles of the methods finally adopted after some months of experimental work may be summarized as follows. The spores, after suitable preliminary treatment with ammoniated alcohol (see p. 197), were mounted in a modified form of acetocarmine squash on glass. After ringing the cover-slip with wax, the field was examined in the usual way with visual light. The selected cell was then transferred to a quartz slide by a method perfected by F. V. Welch and conveniently known by his name (see p. 198). The specimen was then decolourized, mounted under a quartz cover-slip, and examined photographically by ultraviolet light (see p. 199).

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[1] Tradescantia, Sax and Sax, 1935; Lilium, Geitler, 1938; Trillium, Huskins, 1941.
[2] Tulipa, Upcott, 1935.   [3] Osmunda, Manton, 1939.

Apart from the special modifications described in detail in the next section the cytological methods were otherwise those long in use in the botanical laboratories of Manchester University, while the optical methods were essentially those long established at the National Institute for Medical Research. The close contact that there has been between the technical resources of the two institutions is the reason for the presentation of these results as a joint communication. It should, however, perhaps be explained that the scope of the two authors has been somewhat different. One of us (J. S.) has been primarily responsible for the optical methods with ultraviolet light, while the other (I. M.) should alone be held responsible for any statements made as to the cytological meaning of the observations.

MATERIALS AND METHODS

Material. Spores from naturally dehiscing sporangia were collected into envelopes as required from plants growing in the experimental grounds of Manchester University. These plants are the same as those previously used for cytological study of the sporangia and comprised both diploids and triploids. By taking advantage of the seasonal differences found between plants growing in the open and under glass a sequence of fertile fronds is obtainable throughout a season lasting from April till September. The spores must be used fresh and are worthless after about six weeks.

Method of germination. Germination takes place at once if the spores are merely scattered into water at room temperature. Prolonged exposure to direct sunlight should be avoided, but complete shade is not essential. In cold weather the proximity of a radiator is sometimes advantageous. In the summer months (June to August) divisions occur at the rate of one per day, the second division taking place on the second day from sowing, concurrently with the rupture of the outer spore coat and emission of the first rhizoid. Later in the year, or with stale spores, growth is slower and 3 to 5 days may be necessary to reach this stage. The best cytological results are to be expected when growth is rapid.

Treatment for spiral structure. The only special treatment found to be effective for this purpose was to immerse the spores in 50 per cent. alcohol, made alkaline with a drop of strong ammonia, for 5 to 7 minutes before fixing. The cell of Pl. VI, Fig. 6, was obtained in this way. Most of the data to be discussed below, however, were from an exceptional cell which showed its spirals without this pretreatment. It is not possible to determine why this should have occurred, but it is likely to have been due to some physiological consequence of genetical unbalance, since the spore was from a triploid plant and had more than the normal complement of chromosomes (see p. 202).

Methods of mounting. The germinating spores may be examined by sections or by squashes, the former method being useful in the present instance only for some of the preliminary work. Any good cytological fixative is suitable for sections, 2BE (La Cour, 1931) and half-strength chrom-acetic-formalin

being equally satisfactory. Reagents must be changed with a pipette and embedding should be done in a thin glass tube which is allowed to stand in the oven sufficiently long for the spores to sink to the bottom of the molten wax. After the wax has been solidified the glass can be broken away and the layer of spores cut up. Pl. VI, Figs. 1–5, are from sections.

Squashing requires the use of rather more violent methods than are generally necessary. The spores are fixed overnight, or longer, in 1:3 acetic alcohol. A few are transferred to a slide with a pipette and the fixative drained off. A drop of acetocarmine is added and allowed to stand for a few minutes. It is then replenished, a cover-slip is put on, and the slide is heated over a flame. Boiling is not necessary, though it does little harm except to scatter some of the spores away. While hot, the preparation is put down on the table and struck all over with the butt end of a pencil which is allowed to fall upon it under gravity from the height of a few inches. This bursts the cell walls and expresses the protoplasm and nuclei, though even a glass cover-slip is sometimes fractured. Excess liquid is then blotted off by passing a finger once or twice over the surface of the mount after covering it with a piece of filter-paper. This adds the final pressure to the cover-slip which should adhere firmly to the slide without air bubbles beneath. Evaporation is then prevented by ringing.

Method of transfer from glass to quartz. Welch's transfer method will be published in full elsewhere and only the details required here will be mentioned. For an acetocarmine squash the slide and cover-slip are separated and treated at first as if for mounting in balsam by McClintock's method. Thus the wax ringing-material is scraped away, the preparation is soaked in 45 per cent. acetic acid till the slide and cover-slip come apart, and the surface bearing the required cell is transferred through graded mixtures of glacial acetic acid and absolute alcohol in proportions 1:1, 1:3, and 1:9 into absolute alcohol. There is a risk of loss of the specimen in the first movement of the cover-slip, but if it adheres to the glass at this stage it is unlikely to be lost in the transfer process itself. Transit through the alcohols should be slow; for this material a quarter of an hour in each mixture is not too long. From absolute alcohol the surface should be rinsed with butyl or amyl acetate and then flooded with a dilute solution (approximately 1:20 by volume) of commercial Durofix (cellulose nitrate) in one of these solvents. The Durofix impregnates the specimen and on drying for half an hour in a cool oven (an incubator at 37 ° C. was used) it forms a thin film which, on being submerged for a few minutes in water, will spontaneously detach itself from the glass, bringing the specimen with it. Before submerging it, a convenient area of film containing the specimen should be circumscribed by a cut from the tip of a knife or needle; this region will then float up first. The detached piece of film can be floated on to any new surface of glass or quartz to which it will adhere on drying, half an hour at 37° C. again being sufficient. The Durofix can then be dissolved out of the specimen and be replaced, by stages if necessary, by any desired mounting fluid. In the present work the trans-

ferred specimen after removal of the Durofix was decolourized in 45 per cent. acetic acid and then mounted for examination either in that liquid or in the mixture of glycerine and water used for immersing the quartz objective.

Optical methods with visual light. All the usual refinements for visual work with high powers were naturally employed and need scarcely be enlarged upon. The objective was a Zeiss apochromat N.A. 1·4, used with a Watson Holoscopic oil immersion condenser adjusted to critical illumination. Leitz 'Objektol' was substituted for cedar oil as the immersion fluid for the objective, since it does not become viscous on exposure to air, a valuable quality for work on liquid mounts. Illumination was by a parallel beam from a Pointolite lamp screened with Wratten filters B and E (green and orange) for sections and B only (green) for carmine mounts.

Photography was found to be indispensable even with visual light, since for fine details the camera proved to be both more sensitive and more trustworthy than the eye. Ilford Special-Rapid Panchromatic plates were used, the optical arrangements being the same as for direct vision except that the microscope was horizontal and a blue screen was sometimes substituted for the green.

As is well known it is not possible with visual light to exceed a significant magnification of 2,000 diameters under any circumstances and for an object mounted in a liquid of relatively low refractive index, such as acetic acid, this degree of enlargement is likely to be already excessive. Figs. 6 and 7*a–c*, Pl. VI, are selected from a large number of photographs at this magnification, but on balance the results with visual light are outclassed by those obtained with ultraviolet.

Optical methods with ultraviolet light. The ultraviolet photomicrographic apparatus was the horizontal one described and figured by Barnard and Welch (1936), and only those details essential to this work will be given here. With the exception of the objective the optical parts (by Zeiss) are of crystalline quartz. The immersion objective (also by Zeiss) is built up of fused quartz components; it has a numerical aperture of 1·25 and is computed to work in the wave-length of 275 $\mu\mu$. A monochromatic beam of this wave-length is obtained by passing the light from a condensed cadmium spark through a simple monochromator consisting of a quartz collecting lens followed by two 60° quartz prisms. In the cadmium spectrum the 275 $\mu\mu$ line is so well separated from the neighbouring lines that an image of the source can be formed in this wave-length at the back focal plane of the condenser. The immersion fluid for both objective and condenser is a mixture of pure glycerine and distilled water, adjusted by means of a refractometer to a refractive index of 1·447 for the sodium D lines.

An alternative system of illumination by visual light is provided to facilitate the location of the object and the centring of the condenser. The focusing of the condenser and objective in ultraviolet light and the final adjustment of the specimen is then carried out with the aid of a searcher eyepiece placed temporarily over the quartz projection ocular. In it a fluorescent image of

the object is made visible, and if this image is sharply focused, the ultraviolet beams emerging from the projection ocular will form a sharp image on the photographic plate, at approximately the required focal level, when the searcher is replaced by the camera body.

When all adjustments have been made, the observations are obtained by taking a series of photographs at every desired focal level through a specimen. This is made possible by means of the slow-motion fine adjustment (constructed by Messrs. R. and J. Beck) which will effect movements of any desired magnitude down to $0 \cdot 05 \, \mu$.

Photography with the ultraviolet microscope was carried out on Ilford Process plates at an initial magnification of 1,000 diameters, further magnification being introduced in the printing by photographic enlargement. It was found by experiment that the optimum size for making detail visible without blurring the outlines was not attained at less than 3,000 diameters, while for purposes of reproduction 4,000 diameters was often preferable. Both these sizes are represented in the plates. In addition, the differential absorption of the ultraviolet light by the chromosomes is so strong that a more contrasting picture is obtained in this way from an apparently colourless specimen than can be achieved with visual light by the use of stains.

OBSERVATIONS

Scope of the inquiry. In the single imperfect count of one chromosome in the tapetum of Osmunda (Manton, 1939) the number of coils was expressed as 'ca. 14'. This observation, though admittedly inaccurate, nevertheless revealed a striking difference between the length of the chromonema involved in the coils of spiral structure at mitosis and meiosis, the length of thread in both meiotic divisions being much less than at mitosis. This difference in length was traced to a 'supercontraction' which took place in the late prophases of the first (or heterotype) division. The supercontracted state lasted from 'strepsitene' until the end of meiosis, but nothing was known as to when or how recovery took place from it, and it was likewise impossible to determine the numerical extent of the shrinkage until more accurate information was available with regard to the somatic divisions.

The first object of the new work was to fill in these gaps. Numerical data of considerable accuracy are now available regarding the average number of coils, the range of difference between the longest and the shortest chromosomes, and the number of coils possessed by certain recognizable individual chromosomes. This enables the extent of supercontraction to be estimated with reasonable precision and it proves to be a very simple relation. Secondly, the extension of the investigation to the post-meiotic divisions makes it possible to determine the probable time of recovery from supercontraction within close limits, though nothing is yet known as to the manner of it. Lastly, some entirely new ground has been broken by successful observations on direction of coiling not only among different chromosomes in one nucleus but also, in favourable places, among homologous parts of sister chromatids

in the same chromosome. These, while only in a sense preliminary to much further work, are perhaps the most interesting new facts which the investigation has yielded, since they are of a kind which, so far as we know, has not previously been demonstrable in somatic divisions.

Preliminary observations with visual light. Sections of untreated material were used to establish the order of segmentations in the germinating spores and for preliminary comparison with the previously published figures of mitosis in the sporangium. The order of segmentation is that the first mitosis, which takes place immediately on sowing, cuts off a little lenticular cell from the main body of the spore. This small cell divides no further but forms the first rhizoid. The second mitosis (the one to be studied here) takes place in the main body of the spore on the second day from sowing, after the spore coat has ruptured and the rhizoid begun to elongate. The special suitability of this division for spiral structure work is no doubt partly due to the large size of the nucleus, which diminishes later, but also, probably, to the unusual conditions resulting from the recent emergence of the soft inner spore wall.

A median view of a three-celled prothallus at the beginning of the third day is shown in Pl. VI, Fig. 1; the rhizoid projects to the left and parts of the split outer spore coat are visible above and below the two-celled body. Polar and side views of metaphases leading up to this condition are given in Pl. VI, Figs. 4 and 5. A general view and detail of an anaphase are given in Pl. VI, Figs. 2 and 3. Comparison of all these with the corresponding figures in the previous paper (Manton, 1939; Figs. 26 and 47 for anaphases and Fig. 46 for the metaphase) leave little doubt that the general size relations of the chromosomes in the young prothallus resemble those of the tapetum and archesporium and not those of the spore mother cells in meiosis. Recovery from supercontraction is therefore likely to occur immediately on germination of the spore and the number of coils per chromosome in the young prothallus is likely to resemble that previously seen at mitosis in the tapetum.

That this expectation was justified was shown by the preliminary work with visual light on ammoniated material. Pl. VI, Fig. 6, is from a metaphase of the second division in a normal haploid spore obtained in July 1941 with the pretreatment described on p. 197. This specimen is of interest as the first successful example of its kind to be met with, though it is included here chiefly for the comparison which it affords with the cell used later for study with the ultraviolet. The latter (see p. 197 and below) was unusual both in its origin and chromosome number, but in the light of Pl. VI, Fig. 6, there is no reason to suspect any gross abnormality in the structure of the spirals. The cell of Pl. VI, Fig. 6, was not worked out in detail because attention was at that time devoted to analysis of an anaphase which was present on the same slide. In this anaphase counts of coils were made for two chromosomes which were recorded as 'not less than 16 nor more than 17 coils' in each case. The authenticating photographs of this anaphase are not

reproduced here since it is thought unlikely that the significant detail will survive the process of reproduction. Other counts were also made at a number of stages of both the second and the third spore divisions. Enough has been said, however, to indicate the general result which was to confirm the previously published count, 'ca. 14', as of the right order though probably a little too low for an average value.

Some preliminary observations made on direction of coiling with visual light can more conveniently be mentioned in presenting the facts for the ultraviolet.

Observations with ultraviolet. The cell used for ultraviolet study is shown on a small scale in Pl. VII, Fig. 10. It was found unexpectedly in August 1942 in a culture of spores from a triploid plant grown solely for chromosome counts in connexion with another problem. The cell at once attracted attention by the clarity of its spirals which had not been evoked by special treatment. The fact that it had 33 instead of the haploid number of 22 chromosomes was not unusual in cells of triploid origin ($3n = 66$), though no other example of spontaneous revelation of the spirals was met with. The cell was studied repeatedly by visual light at intervals for about two months. Some two dozen exposures were made, the best parts of which are contained in Pl. VI, Figs. 7a–c. In December 1942, the preparation still being apparently in good condition, a transfer was made to a quartz slide. The cell was found to have been stuck to the glass cover-slip and was therefore inverted after transfer. This appeared to be no disadvantage and a series of 60 exposures, known afterwards as the *A* series, was taken right through it at distances of $0 \cdot 1 \mu$ apart. The horizontal area of the plate of chromosomes was unfortunately too large for it to be covered completely by the field of view, and a second series of 30 exposures, known as the *B* series, was taken, $0 \cdot 2 \mu$ apart, to complete the field. Since all these photographs were mirror images of those taken with visual light it seemed both desirable and possibly instructive to turn the object over. This could have been done at once had it been transferred to a cover-slip instead of to a slide in the first instance, but as it was, a second transfer had to be made, this time to a quartz cover. A third series, known as the *C* series, was then taken through it at intervals of $0 \cdot 1 \mu$. When the photographs were printed, it was found that the specimen had shrunk slightly at each transfer (compare for example Pl. VII, Figs. 11 and 12), but was otherwise unaltered. The shrinkage is not necessarily due to the transfer process in itself, but is more likely to be caused by a tendency towards recovery from the reversible enlargement previously induced by the acetic acid, whenever that acid is removed. In this instance the mounting medium for the *C* series was the glycerine immersion fluid (see p. 199), while that of the *A* and *B* series was 45 per cent. acetic acid.

All three series, together comprising 120 significant exposures, are represented in the plates, though naturally only a fraction of the contained evidence can be reproduced. To compensate for this it is proposed to consider in some detail the facts for a few of the most significant chromosomes, notably

Nos. 16 and 19, and to summarize the general results for the rest, adding sample exposures to illustrate some of them. It will facilitate understanding of the selected chromosomes if the bulk of the general results are given first. These can conveniently be subdivided under the headings of chromosome morphology, number of coils, and direction of coiling.

Chromosome morphology. The outline and the position of the spindle attachment (centromere) for every chromosome are given in Text-fig. 1 which
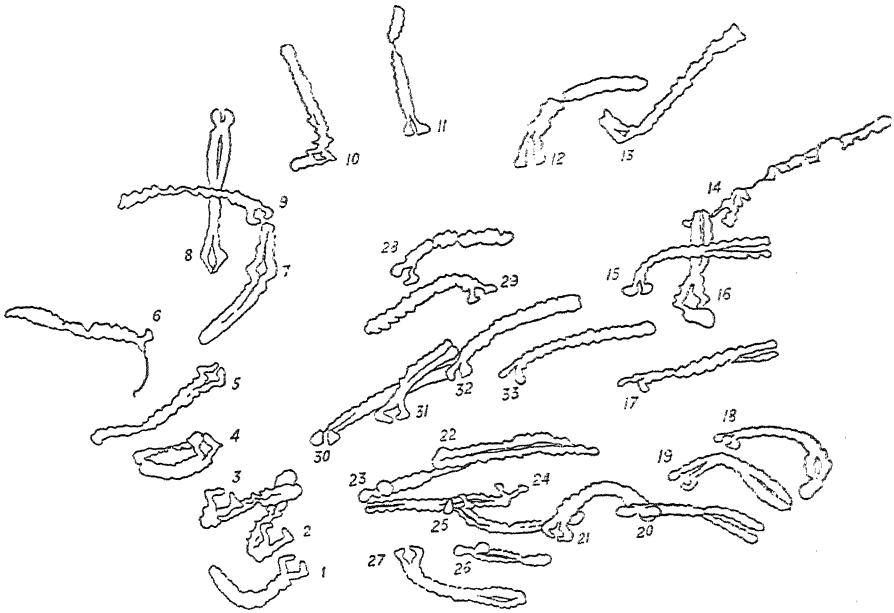
TEXT-FIG. 1. Diagram of the plate of chromosomes used for ultraviolet photography, based on an enlargement of Pl. VII, Fig. 10. The number of each chromosome is placed opposite to the centromere. (Magnification of the diagram, 2,000.)

is reconstructed from the photographic evidence drawn upon the basis of a matt surfaced enlargement of Pl. VII, Fig. 10, which was taken by visual light. Every chromosome is numbered, the number in each case being placed near the centromere (spindle attachment) of the chromosome to which it belongs. The fact that the position of the centromere can be determined with ease in every chromosome present does not yet make possible a description of the morphology of the haploid complement, for the presence of 33 instead of 22 chromosomes in the cell means that many must be present in duplicate. For the present purpose, however, this disadvantage is more than offset by the increased number of chromosomes available for the study of detail.

The importance of determining the position of the centromere in a chromosome is that this is the most obvious character by which identification of individuals can be attempted. In the present instance the great majority of the chromosomes have a spindle attachment so nearly terminal that this end of the split structure has the very characteristic appearance as of the head of a pair of

carpenter's pincers (cf. Pl. VI, Fig. 8; Pl. VII, 11*k*, &c.). In this cell 29 out of the 33 chromosomes present have a short arm (i.e. the piece of chromosome beyond the centromere) of about one coil or less. In two chromosomes, Nos. 10 and 13, the short arm is longer, consisting of 2–3 coils (see Pl. VI, Fig. 9*d*; Pl. VII, Fig. 19). In a further two chromosomes, Nos. 7 and 16, the short arm is longer still and consists of about 6 coils (see Pl. VI, Fig. 9*b* and *c*); these can be counted in chr. 7 but can only be inferred in chr. 16 (Pl. VII, Fig. 11) owing to its attitude; the close resemblance of the long arms in the two strongly supports this inference, however.

The fact that the last two categories of short arms are each exemplified by two chromosomes does not necessarily mean that they would both be present twice in a haploid cell, since both may be examples of duplicated homologues. It is also uncertain to what extent, if any, other distinguishing marks shown by individual chromosomes in this cell, notably the very deep constrictions in chr. 11 and 12 (see Pl. VI, Fig. 9*d* and Pl. VII, Fig. 19), are permanent features of diagnostic value rather than perhaps artificial consequences of the method of mounting. Without analysing further, however, it is possible already to make a very valuable comparison with the results previously published. In Fig. 70 of the previous paper (Manton, 1939) a chromosome with a relatively long short-arm was figured as the best evidence available regarding the number of coils at the second meiotic division. In it 3 very beautiful little coils were shown in one arm and 5 in the other, making 8 in all. This distribution of length between long and short arms can only be matched here in chr. 7 and 16. One or both of these must be the homologue of the previous Fig. 70 and a direct comparison of the same chromosome in the supercontracted and normal states can therefore be made.

Number of coils. The numerical results for the whole plate of chromosomes are summarized in the Table, in which the data from all three ultraviolet series are collated with those from visible light. The chromosomes vary in clarity both in themselves and, to a lesser extent, as they appear in the different views. Even in the best it must be recognized that it is impossible to evaluate a fraction of a coil, and the presence of incomplete coils at either end, or against the centromere, makes an irreducible source of slight error. For the present purposes, however, such error is immaterial. Good examples of the evidence are contained in Pl. VI, Fig. 9, and Pl. VII, Figs. 17–19, &c.

The essential points in the numerical data may be summarized as follows: (1) The commonest number of coils per chromosome is approximately 16. (2) The range of difference between chromosomes includes numbers varying from 14 to 18. These numbers are not likely to be exceeded in either direction to any significant extent in a normal cell. (3) One chromosome, No. 26 in this cell, is unusual in having only about 10 coils (see Pl. VII, Figs. 14*a*, *b*, and 15). Since the previous evidence (see for example Fig. 58 of the previous work) was strongly against there being any one chromosome in the normal comple-ment, of such very distinctive shortness, this one is best interpreted as a chromosome which has broken in the course of meiosis in the triploid, a

<div align="center">TABLE</div>

Data collating Observations on Numbers of Coils per Chromosome in Each of the Three Ultraviolet Series and in Visual Light. The Probable Correct Value is based on the Clearest Observation and is not necessarily the Mean of All the Series. Fractions of a Coil cannot be evaluated and are likely to have been either ignored or treated as One Coil

Chromosome number.	A series.	B series.	C series.	Visual.	Probable correct value.
1.	—	c. 15	—	c. 15	15
2.	—	16–17	—	16–18	17
3.	—	c. 16	—	More than 14	16
4.	—	—	—	18±1	18
5.	—	15	c. 14	15–17	15
6.	—	17	17	16	17
7. Long arm	—	10–11	—	10 ⎫	
Short arm	6	6	—	6 ⎬	16
8.	—	—	c. 18	c. 17	17–18
9.	16	—	—	16	16
10.	18–19	—	18–19	c. 18	18–19
11.	c. 16	—	c. 16	c. 16	16
12.	—	—	c. 15	c. 17	16
13.	c. 18	—	c. 18	c. 15	18
14. Damaged	Count impossible, but not less than 14				—
15.	16–17	—	—	c. 16	16
16. Long arm	10–11	—	10–11	—	
Short arm	?	?	?	?	10+?
17.	—	—	c. 15	c. 15	15
18.	18–19	—	17–19	—	18
19.	16–17	—	16	—	16
20.	Count impossible, but not less than 14				—
21.	Count impossible, but length normal				—
22.	16	—	—	16	16
23.	16	16	—	—	16
24.	c. 15	—	—	—	15
25.	—	—	16–17	16–17	16–17
26. Fragment	—	c. 10	10	—	10
27.	15	16	—	—	15–16
28.	—	—	—	c. 15	15
29.	15	—	14	14	14
30.	—	18–19	c. 18	—	18
31.	c. 14	c. 14	13–14	—	14
32.	c. 17	—	c. 16	—	16–17
33.	c. 17	—	18	—	17–18

happening which is known to occur (Manton, unpublished observations). (4) In the characteristic chromosomes Nos. 7 and 16 the long arms possess about 10 coils, while the short arm in the case (No. 7) which can be counted contains about 6 coils. These numbers are directly comparable with the distribution of 8 coils into two arms with 5 and 3 coils respectively shown by the same chromosome at the second meiotic division. There are, therefore, twice the number of coils in the spiral at a somatic division compared with the state of the same chromosome at the close of meiosis.

Direction of coiling. Observations on direction of coiling were made on twenty different chromosomes in this cell, but they will not all be enumerated in detail since it is the qualitative rather than the quantitative results which must be established in the first instance.

As is well known, the direction of coiling of a spiral may be right-handed or
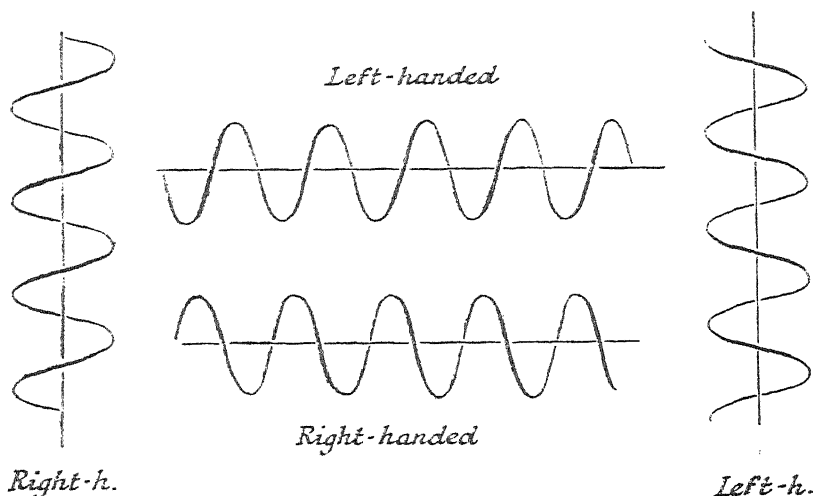
TEXT-FIG. 2. Diagram to show morphology of right-handed and left-handed spirals.

left-handed. A 'right-handed' spiral is so named in the physical sciences[1] after the ordinary carpenter's screw in which the motion of the head is clockwise when the screw is being driven in. A screw of the reversed orientation is 'left-handed'. Direction can be determined, in the case of the screw, by simple inspection of the relative slope of the thread on the front or back surface; with a transparent microscopic object, observation of the change of apparent slope of the lines visible in the top and bottom focal levels is required. This will be obvious from the diagrams in Text-fig. 2. If only one level can be clearly focused, direction cannot be determined with certainty, a fact which limits the number of suitable chromosomes very considerably.

Both directions of coiling can be demonstrated in the plates. The significant part of a figure, where this might be doubtful, is indicated by an ink arrow sloped at approximately the same angle as the gyres of the spiral to which it points. The different focal levels in a sequence are distinguished by letters of the alphabet and are always quoted from above downwards; thus Pl. VII, Fig. 14*a*, belongs to the same sequence as Pl. VII, Fig. 14*b*, but is at a higher focal level.

Examples of left-handed spirals are the lower chromatid of chr. 26 (Pl. VII, Fig. 14*a–b*), the upper chromatid of the horizontal part of chr. 18 (Pl. VII,

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[1] As has been pointed out by Frey-Wyssling (1935) the biological sciences often use these terms in the exactly opposite sense since their idea of motion is often based on the movements of growth of a climbing plant. The usage of the physical sciences will be that adopted here.

Fig. 13*f*–*g*), and both chromatids of chr. 8 (Pl. VI, Fig. 7*a*–*c*), the right side being the clearer in the original prints; Pl. VI, Fig. 7, is of interest as the sole example, because it was the best, of the visual light series.

Examples of right-handed spirals are the inner side of the distal end of chr. 18 (Pl. VII, Fig. 13*a*–*c*), only a short piece being determinable, and both chromatids at the distal end of chr. 16 (Pl. VII, Fig. 11*a*–*d*), which will be considered in greater detail below.

Determination of direction in single short lengths of chromatid in 11 different chromosomes gave 6 as right-handed and 5 as left-handed. These numbers are too few to be statistically significant, but they suggest equality in the two directions.

Greater interest attaches to the observations on changes of direction and on the behaviour of sister chromatids. Changes of direction were demonstrated in six chromosomes. One of these, chr. 18, has already been referred to since opposite ends of one and the same chromatid have been quoted as examples of the two directions. The site of the change cannot be directly observed here, though it is likely to be at the constriction below the marked place in Fig. 13*a* and *c*. The sites of changes of direction can be more clearly seen in chromosomes 6 and 9, both of which are in side view; sample exposures are given in Pl. VI, Fig. 9, and Pl. VII, Figs. 17–18. The most impressive demonstration of change of direction is, however, given by chr. 16, which will now be considered.

Direction of coiling in sister chromatids in the selected chromosomes. The evidence for chr. 16 is so important that it is given in some detail and occupies the whole of the left side of Pl. VII, which the reader is asked to scrutinize with care. Pl. VII, Fig. 11*a*–*k*, are from the *A* series with every alternate exposure omitted, they are therefore 0·2 μ apart. Pl. VII, Fig. 12*a*–*d*, are the corresponding parts of the *C* series, again with every alternate exposure omitted. It may be recalled (p. 202 above) that the *A* and *C* series are mirror images of each other, having been taken from opposite sides of the specimen which also shrank slightly between the taking of the two series. In each column the higher focal levels are towards the top of the page and, to facilitate the reading of the series, ink arrows, sloped at the appropriate angles, are drawn beside the relevant parts of the most significant exposures. Comparison of Pl.VII, Fig. 11*a* and *d* (if necessary with the aid of Text-fig. 2) is a complete demonstration of a right-handed coil in the distal portion of both chromatids. The remaining figures, notably Pl. VII, Fig. 11*f*, *h*, *j*, and Fig. 12*a* and *c* demonstrate equally or more clearly that both chromatids are coiled in a left-handed manner in the immediate neighbourhood of the centromere, the centromere being indicated by a bend in the chromosome. The change of direction occurs simultaneously in both chromatids at the constriction which is visible in all the photographs. The nature of this constriction is not yet fully elucidated.

It is important that the evidence for chr. 16 should be fully understood, for it is the most perfect example of its kind in the whole cell, and without it the

different conditions of the somewhat more complicated case of chr. 19 are unlikely to be apprehended. Out of seven double determinations (i.e. determinations of direction in homologous parts of sister chromatids) five resembled chr. 16 in having their chromatids coiled alike in the regions, sometimes short, which could be analysed. In two cases, on the other hand, sister chromatids were coiled in opposite directions. Of these two cases chr. 19 was the clearer.

The evidence for chr. 19 is contained on the right side of Pl. VII, Figs. 13*a–g* and 14*a–c*, being from the *A* and *C* series respectively, both treated as before as regards spacing and conventions. This chromosome requires somewhat closer scrutiny than is necessary for chr. 16, since the lines are slightly blurred by the presence of a particularly dense investment of cytoplasm which absorbs some of the light and cannot be wholly eliminated in the printing. The slope of the lines, however, is not in doubt.

Taking the more difficult chromatid of chr. 19 first, Pl. VII, Fig. 13*f*, shows the lines on the vertical portion of the outer (right) chromatid to be downwards from left to right. Moving up the series, the slope changes at the tip of this chromatid in Pl. VII, Fig. 13*d* and in the lower portion in Pl. VII, Fig. 13*b*. The distal seven or eight coils in this chromatid are therefore in a right-handed spiral. This direction can be confirmed by comparing Pl. VII, Fig. 14*a* and *c*, in the *C* series, the outer chromatid being in this case on the left-hand side.

The inner chromatid of chr. 19 is fully shown in the *A* series only. Thus in Pl. VII, Fig. 13*c*, which illustrates the upper focus of the last five or six gyres on the left-hand side, the slope of the lines is down from left to right. The change-over to the lower focus in this region occurs in Pl. VII, Fig. 13*e* and *f*; the direction of coiling is therefore in this case left-handed. The distal ends of the two chromatids in chr. 19 are therefore coiled in opposite directions.

The essential features of the demonstration of reversed orientation in sister chromatids of chr. 19 may perhaps conveniently be summarized by Pl. VII, Fig. 16*a* and *b*. In these, two characteristic focal levels have been selected and all irrelevant matter screened away. Chromosome 19 appears isolated on a white background and direction arrows, had they been inserted, would be exactly as in Pl. VII, Fig. 13*d* and *e*. In passing from Pl. VII, Fig. 16*a* to *b* it should be evident that the changes of slope of the topmost three gyres are exactly inverted in the two sides of the chromosome.

That the reversed orientation does not extend for the whole length of the chromosome is indicated by the uppermost exposures of Pl. VII, Fig. 13. Thus in Pl. VII, Fig. 13*c*, two very oblique gyres are visible beside the lower left-hand arrow. These represent the 9th and 10th gyres on the inner chromatid which is showing its lower focus at this point. The upper focus just appears in Pl. VII, Fig. 13*a*. These two gyres are therefore right-handed as in the corresponding place on the other chromatid. A change of direction has therefore taken place in the inner chromatid only.

Surprising as these observations on chromosome 19 may at first appear, they are fully in accord with what had already been detected with visual light,

several examples of reversed orientation having been met with. Compared with earlier observations the unexpected thing about the cell described here was, in fact, not the occasional presence of chromosomes of the type of number 19 but rather the preponderance, in this particular cell, of chromosomes of the type of number 16. Quantitative study of the relative frequency of similar and reversed orientations among the chromosomes of different nuclei would obviously be a matter of considerable interest. It is hoped that this may, in due course, be carried out, though the labour involved will be very considerable. In the meantime the qualitative evidence on direction provided by the analysed cell may be summed up as having shown: (i) that right and left-handed coiling can occur in the same nucleus; (ii) that homologous parts of sister chromatids may be coiled in the same or in opposite directions; (iii) that changes of direction can occur along the length of a chromosome and may involve both chromatids at the same point or may affect one only.

CONCLUSIONS

It is not proposed to discuss these results further here except to point out two of the more theoretical conclusions which emerge from the observations presented.

The numerical data on the number of coils per chromosome are now sufficiently extensive to establish the facts with some degree of finality. This is of value in confirming and amplifying the results previously published and, as an important consequence, makes possible for the first time a reasonably accurate estimate of the extent of supercontraction. The similarity in diameter of a chromosome at mitosis and at the second meiotic division was already sufficiently demonstrated in the previous work. Comparison of the number of coils present at the two divisions is therefore likely to be the most accurate indication yet available regarding the relative lengths of the coiled thread. It has been shown above that there are twice as many coils per chromosome in mitosis compared with those present at the second meiotic division, and therefore the ratio of the normal length of the chromonema to the supercontracted length must be as $2:1$; in other words, the chromonema in the supercontracted state at the meiotic divisions is half as long as it is at a normal cell-division.

With regard to direction of coiling, it would be unwise to generalize or even to interpret in detail until facts on a more quantitative scale are to hand. One tentative conclusion may perhaps be suggested, however. From observations made available in the literature on direction of coiling at the first division of meiosis it has been reported, notably in the case of Trillium (e.g. *T. Kamtchatikum*, Matsuura, 1937, 1941; *T. erectum*, Wilson and Hutcheson, 1941), that when sufficient numbers of coils (10 or more) are present, changes of direction occur among the homologous paired chromosomes in exactly the same manner as here described for sister chromatids in the somatic cells of Osmunda prothalli. It therefore seems probable that, as regards conditions of coiling, mitosis will be found to resemble meiosis more closely than might

perhaps have been expected. It is hoped later to be able to amplify this statement. In the meantime this work is being continued.

SUMMARY

1. Methods have been worked out for studying by ultraviolet light the spiral structure of somatic chromosomes in the young prothalli of *Osmunda regalis*.

2. Counts of the number of coils have been made on thirty-one chromosomes in one cell. The results show that the commonest number of coils is 16. The range of difference between chromosomes of different length includes values varying from 14 to 18 coils, but is unlikely to exceed these figures to any appreciable extent in a normal cell. One exceptionally short chromosome in the cell studied is thought to be a fragment.

3. Some differentiation of morphological types has been detectable by locating the position of the centromere in every chromosome of the cell studied. By this means it has been possible to identify in a somatic division a chromosome homologous with one previously figured for details of spiral structure at the second meiotic division. The number of coils at mitosis is shown to be exactly double.

4. The ratio of the normal to the supercontracted chromonema length is therefore 2:1.

5. Observations on direction of coiling in twenty chromosomes in the cell studied have shown the following qualitative relations: (*a*) right-handed and left-handed directions both occur; (*b*) homologous parts of sister chromatids can be coiled in the same, or in opposite directions; (*c*) changes of direction can occur along a chromosome arm and may involve both chromatids at the same point or one only.

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DESCRIPTION OF PLATES VI AND VII

Illustrating Dr. I. Manton and Mr. J. Smiles's paper on 'Observations on the Spiral Structure of Somatic Chromosomes in Osmunda'.

PLATE VI

Untouched photomicrographs, Figs. 1–7 taken with visual light, Figs. 8 and 9 with ultra-violet light.

Fig. 1. General view of a median longitudinal section of a three-celled prothallus, fixed in 2BE, stained in haematoxylin and bismarck brown. ($\times$ 500.)

Fig. 2. L.S. of an anaphase of the second division in a germinating spore, fixed in 2BE, stained gentian violet. A portion of the rhizoidal cell resulting from the previous (first) germination division visible at bottom left. ($\times$ 1,000.)

Fig. 3. Detail of single chromosomes from preceding. ($\times$ 2,000.)

Figs. 4–5. Metaphases of the second spore division from the same slide as the preceding. ($\times$ 2,000.)

Fig. 6. Metaphase of the second spore division after pretreatment for spiral structure, mounted and photographed in acetocarmine. ($\times$ 2,000.)

Fig. 7. Three focal levels of chromosome 8 in the cell used for ultraviolet. Fig. 7a the upper focus, Fig. 7c the lower. Photograph in acetocarmine by visual light. ($\times$ 2,000.)

Fig. 8. Ultraviolet photograph of two chromosomes showing terminal spindle attachments, from the *B* series. ($\times$ 3,000.)

Fig. 9. Sequence from the *B* series at focal levels 0·4 μ apart (exposure numbers 8·1, 7·1, 6·2, 5·3). Fig. 9a the highest focus, Fig. 9d the lowest. ($\times$ 3,000.)

PLATE VII

Untouched photographs taken with ultraviolet light except Fig. 10 which is with visual light. The numbers of the chromosomes as in Text-fig. 1, p. 203. Magnification $\times$ 4,000 except where otherwise stated.

Fig. 10. General view of the cell used, taken with visual light, in acetocarmine before transfer to quartz. The arrows point to the principal regions shown in detail elsewhere in the plate. ($\times$ 1,000.)

Fig. 11. Sequence from the *A* series giving evidence for direction of coiling in chromosome 16, the critical observations of slope being shown by arrows. In each column the highest focal levels are towards the top of the page and in the whole series Fig. 11a is the highest and Fig. 11k the lowest focus. Exposures taken 0·2 μ apart (serial numbers 18·2, 17·3, 17·1, 16·2, 15·3, 15·1, 14·2, 13·3, 13·1, 12·2).

Fig. 12. Short sequence from *C* series giving confirmatory evidence for chromosome 16, exposures 0·2 μ apart (serial numbers 13b, 12b, 11b, 10b). Fig. 12a the highest and Fig. 12d the lowest.

Fig. 13. Sequence from *A* series giving evidence for direction of coiling in chromosomes 18 and 19. Exposures 0·2 μ apart (serial numbers 17·3, 17·1, 16·2, 15·3, 15·1, 14·2, 13·3). Fig. 13a is the highest focus and Fig. 13g the lowest.

Fig. 14. Short sequence from *C* series giving confirmatory evidence for direction of coiling in the outer (left) chromatid of chromosome 19, and for the fragmentary chromosome 26. Exposures 0·2 μ apart (serial numbers 7b, 6b, 5b). Fig. 14a the highest focus, Fig. 14c the lowest.

Fig. 15. Demonstration of the shortness of the putative fragment, chromosome 26, in relation to other neighbouring chromosomes of normal length, from the A series. ($\times$ 3,000.)

Fig. 16. Two selected levels of the *A* series to show opposite directions of coiling in sister chromatids at the distal end of chromosome 18. Exposures 0·3 μ apart (serial numbers 16·1 and 15·1). Fig. 16*b*, the lower focus, is the same as Fig. 13*e*; Fig. 16*a*, the upper focus, comes in between the levels of Fig. 13*c* and *d*. In both Fig. 16*a* and *b* the neighbouring chromosomes have been screened away; the slope of the lines is as indicated by the arrows in the adjacent Fig. 13*d* and *e* and they demonstrate a left-handed and a right-handed coil in the top three gyres of the inner (left) and outer (right) chromatids respectively.

Fig. 17. Portion of the *A* series showing parts of two chromosomes, chromosome 9 of interest for a change of direction of coiling along the length of the arm and chromosome 7 important for the position of the centromere (see text and compare with *B* series in Plate VI, Fig. 9).

Fig. 18. Portion of *A* series showing chromosome 6, of interest for a particularly clear count of the number of coils and for a change of direction along its length.

Fig. 19. Portion of *C* series showing morphology of two chromosomes, chr. 12 of interest for terminal centromere and deep median constriction and chr. 13 for characteristic subterminal centromere comparable with that of chr. 10 (Pl. VI, Fig. 9*d*). (×3,000.)
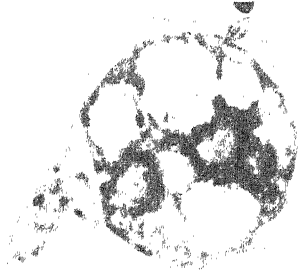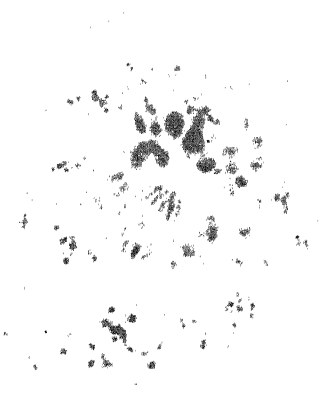
1

2

3

4

5

6

chr.8

7a

7b

7c

chr.29

8

chr.28

chr. 7

chr. 10

chr. 9

chr. 11

chr. 10

chr. 8

chr. 6

chr. 8

chr. 16

11a

11g

12a

11b

12b

11h

11c

11i

12c

11d

11j

12d

11e

chr. 16

chr. 15

chr. 16

chr. 32

chr. 33

chr. 31

chr. 22

chr. 26

13

13a

chr. 19 chr. 18

13b

14a

13c

chr. 20 chr. 21

chr. 26

chr. 18 chr. 19

14b

13d

14c

16a 16b

13e

17 chr. 9

chr. 7

chr. 6

18

13f

chr. 13

chr. 12
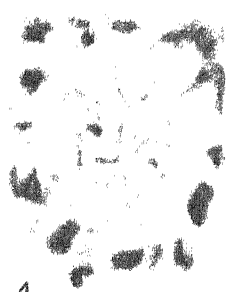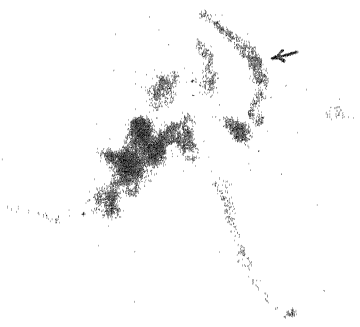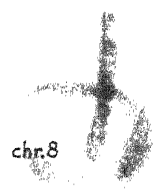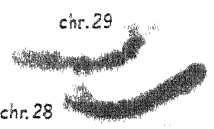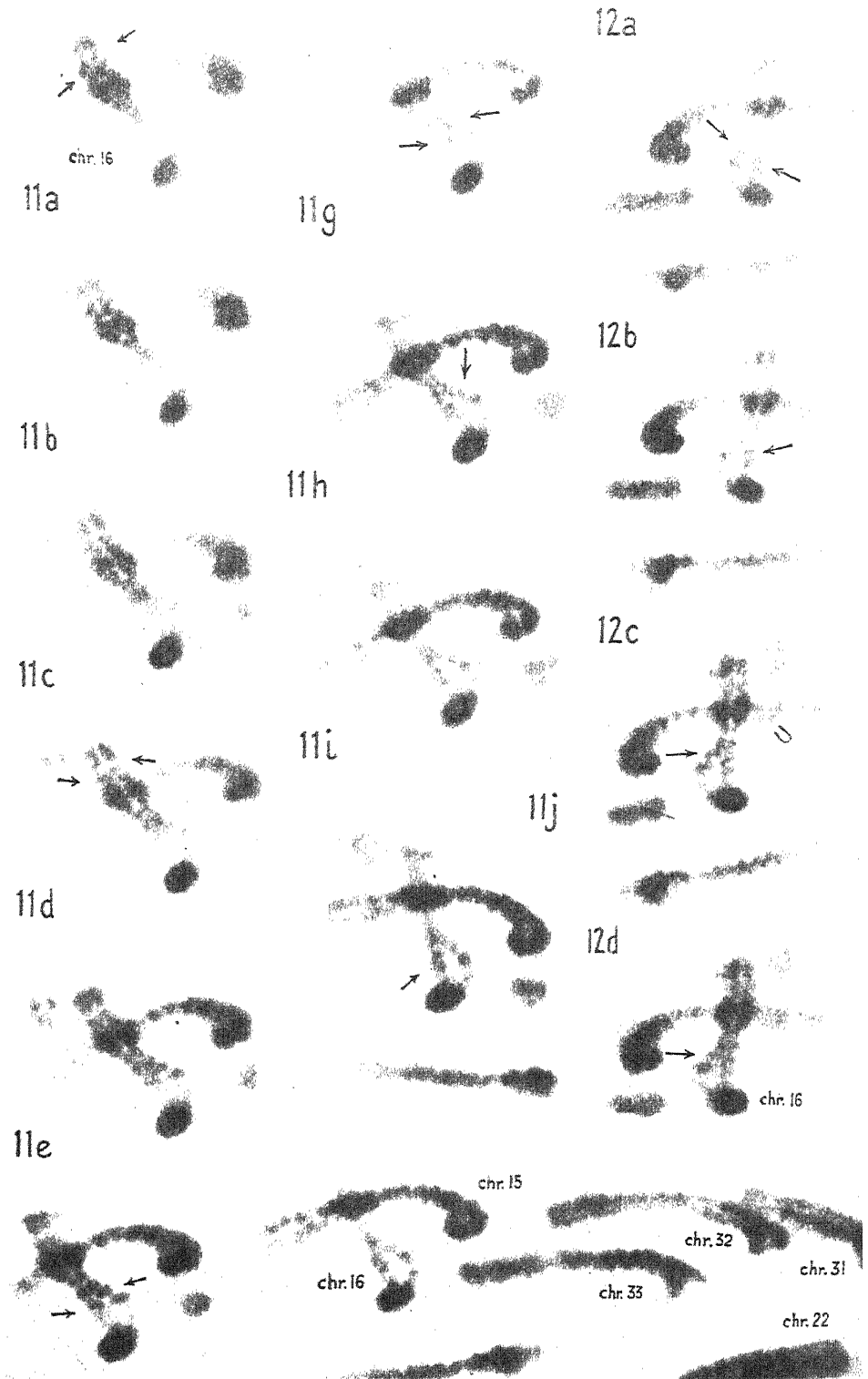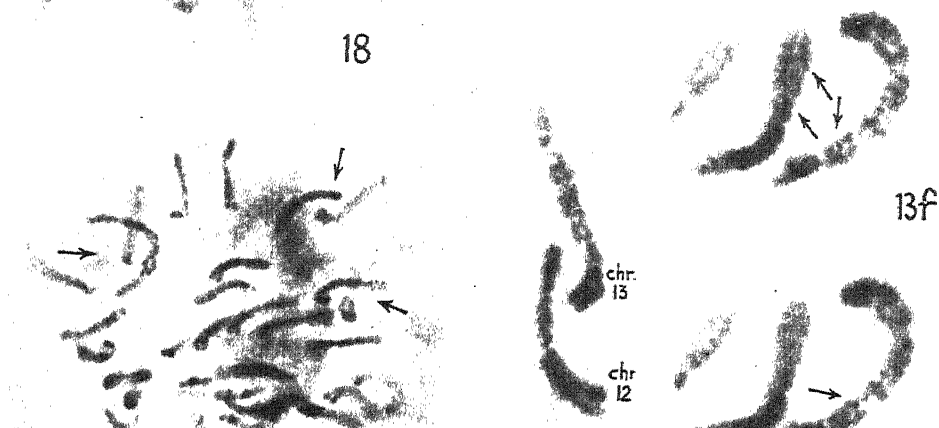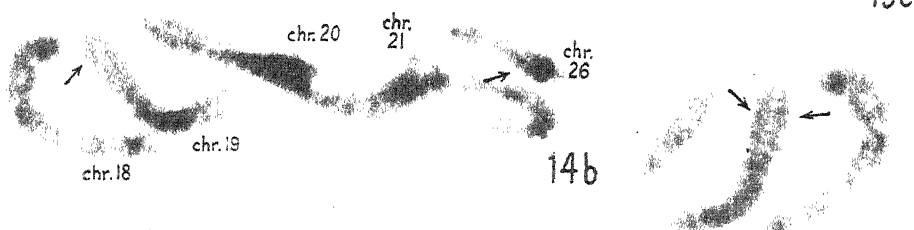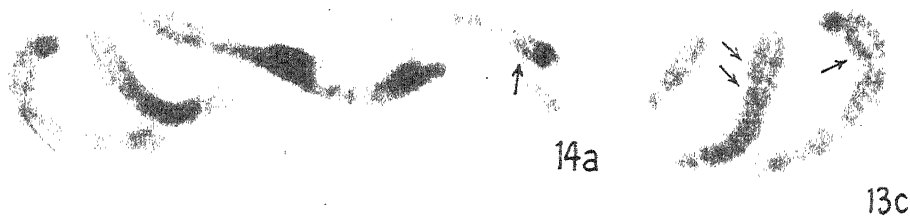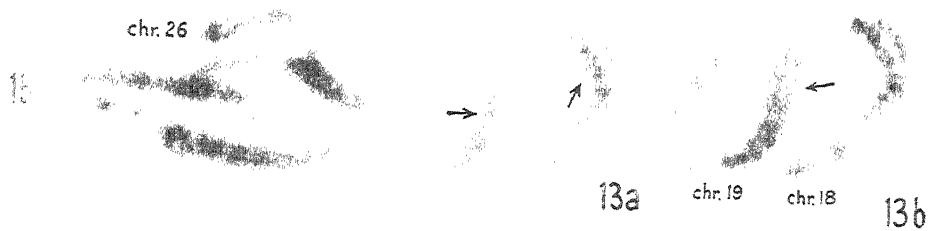
Catenulopsora, a new Genus of Rusts

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With eight Figures in the Text

IN a collection of fungi made at Bangalore in 1940 was a rust on *Flacourtia sepiaria* Roxb. which appears to be new to science. Uredial and telial sori form yellowish-brown patches on the leaves. The former occur throughout the year, but the telia begin to appear in September and persist until the following February.

The uredia are amphigenous but mostly epiphyllous, minute, sparse, and form concentric rings (Fig. 1). They arise subepidermally, but burst through the epidermis at maturity. The urediospores are egg-shaped, light yellowish-brown, and echinulate. They are provided with a single indistinct germ pore which can be seen if the spores are boiled in lactic acid. The pores become more prominent at the time of germination. The urediospores are borne singly on short, hyaline pedicels and are intermixed with paraphyses. Some of the latter lie at the periphery of the uredium, where they are slightly curved (Fig. 2). They do not arise from peridium-like structures at the base, nor do they form pseudoperidia; they are borne directly on the stroma.

The earlier formed telia arise within the uredia, but later in the season separate telia, which are hypophyllous, are produced. A plectenchyma is formed beneath the epidermis, where there is a palisade of vertically arranged hyphal cells, the telial initials. These are binucleate. Later they divide forming two cells, the lower on further elongation becoming the pedicel and the upper developing into a teliospore mother-cell. A series of teliospores are then abstricted (Figs. 3 and 4), from the upper cell, and a filiform chain is formed which increases in length as new spores are produced. Up to 23 spores have been counted in a chain.

The spore chains are slightly waxy, closely arranged side by side, and spread apart even to the hymenial point of attachment in the sorus, without any tendency to lateral agglutination (Fig. 4). At their upper ends the chains are intertwined into a tangle, but this can be easily separated. A few cylindrical paraphyses which are faintly brownish surround the telial sorus. The teliospores are produced in basipetal order, the distal ones being the oldest.

When young, the teliospores are dikaryotic, but the nuclei fuse at maturity. If pear-shaped, sessile, and thin-walled teliospores of some Uromyces were superimposed on one another to form a chain, such a chain would resemble the catenulations formed by the teliospores under study. The spores in a chain

FIG. 1
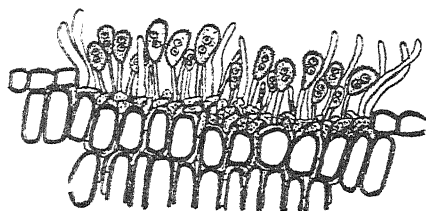
FIG. 2

FIG. 1. Twig and leaves of *Flacourtia sepiaria* showing circinate uredia and telia in the centre.

FIG. 2. Uredium of *Catenulopsora Flacourtiae* showing stipitate urediospores and paraphyses. ($\times 260$.)

firmly adhere to one another, and, though the chains may fall off, the spores do not separate.

The teliospores are thin-walled, yellowish, rectangular at first but globose to pyriform later. If sufficient moisture is present, they germinate without a rest period; spores collected after a rain are usually empty, germination having already taken place. The teliospores are without germ pores, but a prominent beak develops at the upper end which gives the spore a pyriform shape. At the time of germination this beak elongates, curves upwards (Fig. 5), reaching a length up to 95 μ, and at its upper end forms the promycelium. It is unseptate for a greater part of its length (Fig. 6), but nearer the apex the promycelium becomes swollen. The fusion nucleus then migrates into the developing promycelium where two successive divisions occur, leading to the formation of four cells. Each cell of the promycelium then forms a sterigma on which develops a single, smooth, globose sporidium capable of germinating *in situ*. Stained preparations of sporidia show that they are primarily uninucleate, but some may become binucleate by a precocious division of the nucleus and a delay in the formation of septa (Fig. 7).

The long chains of teliospores without germ pores, each with an apex which first develops a beak, and then continues to grow into a long and gracefully curved promycelium, separate this rust from the existing genera of the Uredinales. A new genus has therefore been established for its reception, and the name Catenulopsora is proposed.

In addition, the rust on *Ampelocissus latifolia* Planch. (=*Vitis latifolia* Roxb.) which Butler (1912) named *Chrysomyxa Vitis* but which Sydow (1915) transferred to Kuehneola, and Arthur (1917) to Cerotelium, belongs to the genus Catenulopsora. The urediospores of this rust are intermixed with
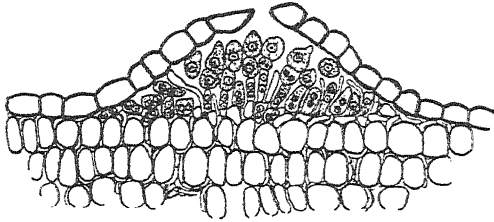
FIG. 3. Young telium. (×260.)
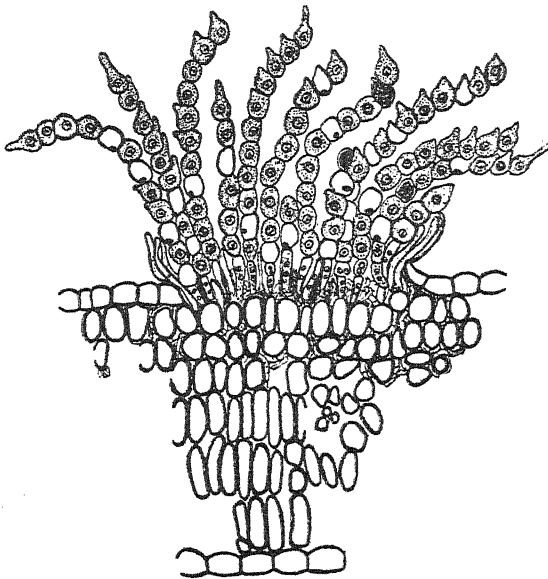
FIG. 4. A more advanced telium showing chains of pyriform
teliospores and paraphyses. (×260.)

paraphyses, and there is a marginal ring of short, incurved paraphyses which arise directly from the stroma. Much stress is laid by Mains (1921) on the presence of a short, delicate peridium in the uredium of Cerotelium from which the paraphyses arise, and on the hyphoid paraphyses that form a pseudo-peridium. Both these characters are absent in the rusts on Flacourtia and Ampelocissus. Butler states that the urediospores are sessile, without germ pores, and that they appear to arise in chains. In material recently collected at Cuttack and also in the type material short and rather indistinct pedicels have been observed, on which the urediospores arise singly and not in chains. If

the spores are boiled in lactic acid, a single rather obscure germ pore can also be seen. Germination by elongation of the spore apex clearly distinguishes the rust on Ampelocissus from Cerotelium and shows that it belongs to a different tribe from the Ochropsorae in which Cerotelium is placed by Dietel (1928).

The teliospores of the rust on Ampelocissus have been found only in the type material, which is rather old and brittle and therefore difficult to cut. They are in tenacious chains without any tendency to lateral agglutination. The chains are not, however, as long as those of the rust on *Flacourtia sepiaria* and they contain up to 6 spores. The germination of the two rusts is, however, similar, that is by the prolongation of the apical region of the teliospore into a tube, of which a major portion is unseptate and which, at the apex, forms a lightly swollen, 4-celled promycelium.

In sections of the type material of the Ampelocissus rust structures which look like pycnia have been noticed below the epidermis. If they are in fact pycnia, then these are subepidermal as against the subcuticular pycnia of Cerotelium and Kuehneola.

Catenulopsora Mundkur gen. nov.

Pycnia, cum adsunt, sparse distributa, subepidermalia. Aecia ignota. Uredia subepidermalia, erumpentia; paraphyses cylindricae, intermixtae urediosporis, annulum marginalem formantes, moderate incurvae. Urediosporae singulae, uno germinationis poro praeditae, brevibus pediculis insident. Telia primo subepidermalia, tum erumpentia, paraphysata, catenarum longarum instar disposita, singulae catenae lateraliter ab aliis disjunctae teliosporarum numero ad 23 constantes. Teliosporae in catena firmiter inter se conjunctae, inseparabiles vel in maturitate; spora basica pedicellata; teliosporae germinationis poris carentes, germinant statim incremento regionis apicalis crescente in longum, productum promycelium catenae parallelum. Promycelium 4-cellulatum in extremo superiori; sporidia globosa sterigmatibus insidentia.

Species typica: *Catenulopsora Flacourtiae* super *Flacourtiam sepiariam* Roxb.

Pycnia, if present, sparse, subepidermal. Aecia unknown. Uredia subepidermal, erumpent; paraphyses cylindrical, intermixed with urediospores, also forming marginal ring, slightly incurved; urediospores borne singly on short pedicels with a single germ pore. Telia at first subepidermal, then erumpent, paraphysate, forming long chains, chains not laterally united, up to 23 teliospores in a chain; each teliospore firmly united to the one below, not separable even at maturity, basal spore pedicellate; teliospores without germ pores, germinating at once by continuation in growth of the apical region into a long, elongate promycelium parallel to the spore chain; promycelium 4-celled at upper end, with globose sporidia on sterigmata.

Type species: *Catenulopsora Flacourtiae* on *Flacourtia sepiaria* Roxb., Bangalore.

Catenulopsora Flacourtiae Mundkur and Thirumalachar, spec. nov.

Pycnia atque aecia ignota. Uredia epiphylla, raro amphigena, minuta, sparse distributa, annulos concentricos formantia in luteo-brunneis maculis; paraphyses cylindricae, tenuiter brunneae, tum intermixtae tum in peripheria; paraphyses in peripheria moderate incurvae. Urediosporae singillatim pediculis insident, ellipsoideae vel obovatae, echinulatae, luteo-brunneae, germina-
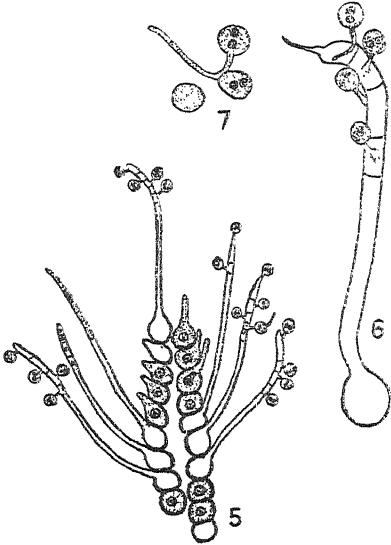
FIGS. 5–7. Fig. 5. Chains of germinating teliospores with sterigmata and sporidia. (×260.) Fig. 6. A germinating teliospore with promycelium, sporidia, and nuclei which have divided precociously. (×550.) Fig. 7. Germinating sporidia. (×550.)
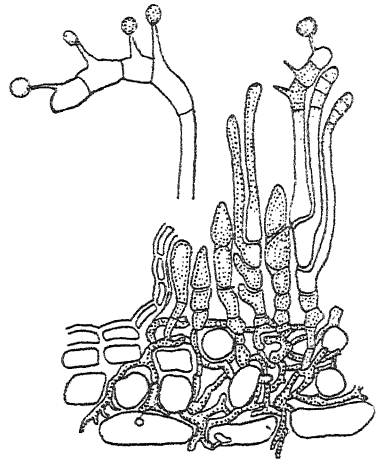
FIG. 8. Germinating teliospores and promycelium of *Catenulopsora Vitis*. (After Butler, Ann. mycol., 1912, p. 157.)

tionis poro indistincto, 13·6–15·4×9·3–13·7 μ. Telia amphigena, plerumque hypophylla, subepidermalia, erumpentia, minuta, sparse circum uredia distributa, aureo-lutea, peripheralibus paraphysibus praedita. Teliosporae in columnis leviter cereis, sporae in catena ad 23 conjunctae. Teliosporae primo sphericae, in maturitate vero pyriformes, catenulatae, singulae firmiter inferiori conjunctae, numquam separabiles, spora infima pediculo insidens, muro tenui praeditae, magnitudinis 18·3–19·4×12·7–14·5 μ, exclusis germinationis poris; statim germinant prolongato superiori rostro in productum promycelium ad magnitudinem 95×6 μ, ut plurimum non septatae, in apice vero 4-septatae; cellulae singulae sporidium efformant globosum, tenui rasoque muro praeditum, diam. ad 7 μ, sterigmati insidens.

Habitat in foliis vivis *Flacourtiae sepiariae* Roxb. leg. M. J. Thirumalachar in loco Yashvantpur prope Bangalore mensis Dec. die 28, 1940. Typus in Herb. Crypt. Ind. Orient. New Delhi positus.

Pycnia and aecia unknown. Uredia epiphyllous, rarely amphigenous,

minute, sparse, forming concentric rings on yellowish-brown patches, with brownish, cylindrical paraphyses both intermixed and peripheral, the latter slightly incurved; urediospores arising singly on pedicels, ellipsoid or obovate, echinulate, yellowish-brown, with an indistinct germ pore, 13·6–15·4×9·3–13·7 μ. Telia amphigenous, mostly hypophyllous, subepidermal, erumpent, minute, sparsely distributed around uredia, golden yellow, with peripheral paraphyses; teliospores forming slightly waxy columns, up to 23 spores in a chain, chains falling apart even to the point of attachment, never laterally jointed; teliospores spherical in early stages, pyriform at maturity, catenulate, each joined firmly at base to spore below, never separable, basal spore on a pedicel, thin-walled, measuring 18·3–19·4 × 12·7–14·5 μ without germ pores; germinating at once by prolongation of upper beak into an elongate promycelium up to 95 μ long and 6 μ broad, unseptate for major portion but 4-septate at apex, each cell forming a thin-walled, globose, smooth sporidium up to 7 μ in diameter on a sterigmata.

Hab. in living leaves of *Flacourtia sepiaria* Roxb. at Yashvantpur, near Bangalore, Dec. 28, 1940, leg. M. J. Thirumalachar. Type deposited in Herb. Crypt. Ind. Orient. New Delhi.

Uredo Uguressae Petch (1909) on *Flacourtia ramontchi* Sher. has uredia without paraphyses and urediospores with large blunt spines and much larger size (24–28×15–19 μ). The uredial stage of *Catenulopsora Flacourtiae* does not agree with that fungus.

Catenulopsora Vitis (Butler) Mundkur and Thirumalachar comb. nov. Syn. *Chrysomyxa Vitis* Butler.

Pycnia subepidermal. Uredia hypophyllous, fairly minute, subepidermal, erumpent, numerous, yellowish, pulverulent; paraphyses hyaline to slightly brownish, cylindrical, slightly incurved, peripheral, also intermixed with urediospores; urediospores globose, obovate, or elliptical, pale yellow, echinulate, arising singly on short pedicels, 17–24×14–20 μ, with epispore 1·5 to 2·0 μ thick and one rather obscure germ pore. Telia hypophyllous, subepidermal, erumpent, more or less aggregate, arranged concentrically, brown, slightly powdery at maturity; teliospores catenate in small tenacious chains each containing 3 to 6 spores, pale brown, each joined firmly at base to spore below, basal spore on a pedicel, thin-walled, ovate to oblong, measuring 11·3–20·4×7·8–11·3 μ, without germ pores; germinating at once by elongation of apical region into a promycelium, up to 75 μ long and 6 μ broad, unseptate for a major portion, but 4-septate at apex, forming four promycelial cells each with a sterigma, 10 μ long, bearing a globose to elliptic sporidium 6–9 μ diameter.

Heab. in living leaves of *Ampelocissus latifolia* (Roxb.) Planch. (=*Vitis latifolia* Roxburgh), Rangpur, October 18, 1909, leg. S. N. Mitra, type deposited in Herb. Crypt. Ind. Orient. New Delhi; Dacca, October 7, 1910, leg. A. L. Som; on *Vitis adnata* Wall (*Cissus adnata* Roxb.) Noakhali, December 7, 1911, leg. E. J. Butler; December 8, 1913, leg. Inayat.

Catenulopsora Vitis appears to be brachyform. It differs from *Catenulopsora Flacourtiae* in having much larger urediospores but smaller teliospores with fewer spores in a chain. There do not appear to be any paraphyses in the telium of the former. It may be noted that Arthur (1917) transferred the Ampelocissus rust which Sydow (1915) had placed in Kuehneola to Cerotelium without seeing a specimen, but it is doubtful if he would have done so had he seen a specimen of the rust.

Another rust whose telia are in the form of worm-like tendrils of horny consistency, capable of swelling in water, is found at Delhi on *Flueggea microcarpa* Blume. It agrees with *Masseeella Flueggeae* Sydow on *Flueggea virosa* Baill. in the Philippines. This rust has now been shown by Sydow and Petrak (1928) and Cummins (1937) to be an aut-eu-form, though pycnia, aecia, and uredia are not known in the type species of the genus, *Masseeella Capparidis* (Hobs.) Dietel. The uredia are subcuticular and without paraphyses as compared with the subepidermal and paraphysate uredia of *Catenulopsora Flacourtiae;* the telia of Masseeella arise in a deeply sunk cavity in the leaves and the teliospores are without pedicels; their walls are very thick and longitudinally finely striate and they have an apical germ pore. A continuous formation of teliospores takes place, and these are held together in a slimy material which when dry forms columns of horn-like consistency. The teliospores are not, however, concatenate and the spore tendrils do not bear any resemblance to the spore chains of *Catenulopsora Flacourtiae*.

Another genus of rusts whose teliospores form chains is Arthuria Jackson (1931). It is characterized by semi-waxy telia whose teliospores are likewise catenulate, there being 3 to 8 spores in a chain. The teliospores are thin-walled and germinate without a rest period, but the germination is not of the unusual type observed in species of the genus Catenulopsora; the germ tubes are, moreover, very short. It further differs from Catenulopsora in possessing catenulate urediospores with intercalary cells, and in the uredia and telia being without peridia or paraphyses. The combination of characters present in the genus herein proposed is widely different from those of Arthuria.

Among the other rusts whose teliospores form concatenate chains and which can be compared with the genus Catenulopsora are Chrysomyxa, Baeodromus, Gambleola, Kuehneola, and Cerotelium. In Chrysomyxa the uredium has a rudimentary peridium and the urediospores are catenulate. The chains of teliospores are longitudinaly as well as laterally united and they possess indistinct germ pores. In Baeodromus, a typically microcyclic genus, the telia are aecidioid and the spore chains are laterally agglutinated. The teliospores with an apical germ pore germinate only after a period of rest and form a typical basidium. In Gambleola, which is another microcyclic genus, the telia form dry, gelatinous, tendril-like columns of teliospores. These teliospores are 2-celled with two apical germ pores in the upper and two lateral ones in the lower cell.

With regard to Kuehneola and Cerotelium, the limits of these two genera are somewhat uncertain. Dietel (1912, 1928) recognizes Kuehneola only on

Rosaceae and states that the uredia are without paraphyses and urediospores without germ pores; but Arthur (1934) does not, and Sydow (1915) apparently does not, accept the limitations placed on the genus by Dietel (l.c.). In any case, the teliospores of Kuehneola have an apical germ pore through which the typical promycelium is exserted, and it is different from the promycelium of Catenulopsora, where the beak in the apical region itself becomes a pro- mycelium by elongation. In Cerotelium the uredia have either a delicate peridium composed of more or less evanescent cells or hyphoid paraphyses united at their bases forming a pseudoperidium; furthermore, the urediospores are sessile. The short columnar telia become pulverulent at maturity and the end cells of the spore chains readily fall away. In addition, the chains are more or less united laterally, and there is a tendency among them to form crusts. The characters which the genus Catenulopsora possesses are different from those of the above genera. The catenulate manner of origin of the teliospores indicates the position of the genus in the tribe Pucciniosporeae of Dietel (1928).

SUMMARY

A new genus named Catenulopsora has been established to accommodate a rust on *Flacourtia sepiaria* found at Bangalore. The teliospores are catenu- late and form long chains which are not joined laterally; the teliospores in the chain are firmly fixed to each other and they do not separate even at maturity. They are without germ pores and germinate without a rest period by the pro- longation of the apical region into a long, curved promycelium which at its apex is 4-septate. Only uredia in addition to telia have been seen on this host.

The rust on *Ampelocissus latifolia*, named *Chrysomyxa Vitis* by Butler, also belongs to this genus. It shows germination characteristic of the type species, and in addition to uredia it also possesses subepidermal structures which are probably pycnia.

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The Absorption and Accumulation of Solutes by Living Plant Cells

X. Time and Temperature Effects on Salt Uptake by Potato Discs and the Influence of the Storage Conditions of the Tubers on Metabolism and other Properties

With fourteen Figures in the Text

1. GENERAL INTRODUCTION

THE object of previous papers of this series[1] has been to describe the salient features of the process of salt absorption by living cells and the effect of the variables by which it is determined. Though devoted largely to storage tissues the work has been presented in its relation to the general problem of salt absorption by plants (see also Steward, 1935, 1937), and emphasis has been placed on the need for recognizing the importance of growth and metabolism as factors in absorption whether by storage tissues or by complete plants.

Stiles and Skelding (1940), however, in opening a new series, on the same subject, appear to adhere to the standpoint of earlier writings (Stiles, 1919–27) in which much less account was taken of the vital nature of the experimental material. Since 1919 the work of Osterhout and others on Valonia and on artificial systems, papers from Hoagland's laboratory upon Nitella, Elodea, and root systems, the contributions of the schools led by Lundegårdh and Collander respectively, a group of writings with which the names of Briggs and Petrie may be linked, and in some measure the standpoint developed in this series, have all contributed to a change in the outlook on problems of salt uptake and the way in which these are formulated for solution and approached experimentally.

[1] The earlier papers (I–IX) of this series are listed in full in the bibliography at the end of this paper. When a reference is made to one of these it will be cited by the roman numeral only. References to papers not part of this series are made in the usual manner.

Stiles and Skelding, however, deal with the 'present status of knowledge' and the 'factors affecting salt absorption' almost as though this fertile period had not been. They make it appear that quantitative experiments are still a novelty in this field and, while crediting some authors with isolated experiments, that systematic investigation has been lacking. To one familiar with the history of the subject the omission and want of appreciation of established facts are conspicuous. Stiles and Skelding's quotations from earlier papers of this series suggest that their readings of these have been superficial, and where this has led to misunderstanding their statements have been corrected.

Many of the essential conclusions of this series have now veen verified and applied to other absorbing systems (Hoagland and Broyer, 1936; Rosenfels, 1935; Prevot and Steward, 1936). Agreement between the results of these investigations arises from attention to certain factors in the technique and recognition of the rôle of growth and metabolism in salt accumulation. Understanding of the process necessitates quantitative measurement of both the salt absorbed and the metabolism of the tissue.

In every investigation on salt accumulation certain factors, e.g. nature and concentration of the external solution, are obvious at the outset and usually need no special methods of control. Temperature almost always affects the rate of physiological processes and its effect in the earlier work of this series did not pass unnoticed. An early experiment was described (I) which emphasized the great effect of temperature on absorption and stands as the justification for the choice and control of temperatures in this series. Work on storage tissue, however, involves variables for the control of which special provision must be made in the design of the apparatus and the conduct of the experiments. This is especially true of all those factors which determine the supply of oxygen to the tissue (aeration, the size, form, and number of the cut pieces of tissue, volume and surface of solution, &c.).

Stiles and Skelding still dispute the value in salt absorption experiments of continuous aeration of the solutions by means of a rapid stream of washed, carbon-dioxide free, air, and criticize both the flowing air technique and the conclusions which have been derived by its use.

Oxygen supply cannot remain uncontrolled if results of experiments are to be accurately duplicated. Confidence that the degree of aeration necessary for maximum salt uptake has been achieved demands an excess of oxygen, which in our view is best ensured by a rapid stream of carbon-dioxide free air (or mixtures still richer in oxygen) passed through the solution continuously. This need is quite apart from the utility of the gas stream in the determination of respired carbon dioxide, although the removal of this product of metabolism also contributes to the maintenance of salt uptake.[1]

It will be recalled that in the apparatus described (II) and used in this series

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[1] The effect of the partial pressure of carbon dioxide in the flowing gas has been investigated. The results have been published in another series of papers on the Biochemistry of Salt Absorption (see *Plant Physiology*, xvi. 481–519, 1941).

the aeration is provided by the above means, 40 to 60 discs (40–45 gm.) of tissue being immersed in 2 litres of continuously stirred and aerated solution in a 4-l. vessel.

In the conditions now advocated by Stiles and Skelding it is clear that the storage tissue (carrot) cannot absorb enough oxygen from the solutions provided and the authors attempt to overcome this limitation in three ways. (*a*) In what is described as 'the usual routine', the bottles are provided with a small aperture and, by shaking, gaseous interchange between the *laboratory air* and the container, as well as between the gas phase and the solution, is facilitated. (*b*) Alternatively, the bottles are periodically opened to air during sampling operations, or (*c*) the bottles are aerated (presumably by laboratory air) from a pump at an unspecified rate for 10 minutes in every hour, and in the interim are unshaken and unstirred.

Similar procedures were used in the preliminary investigations (I) which led to the technique (II) now criticized by Stiles. For potato discs gross shaking must be used with caution since there is a limit to the mechanical agitation which the tissue will tolerate (I). Even so, shaking with an outlet to the air was tried but it was found that the flowing air method, which Stiles and Skelding do not appear to have used in their latest work, was more satisfactory. A factor in the choice of the flowing air method was the knowledge that, if air unpurified entered the vessels, its composition was uncontrolled. Laboratory air is not usually of the requisite degree of purity for physiological investigations.

Stiles and Skelding's comment is that their three treatments, none of which was identical with the technique used in this series, gave results with carrot tissue which were not very different from one another, but the rate of uptake of the intermittently aerated cultures was, if anything, less than those continuously shaken (Stiles and Skelding, 1940, p. 344). These experiments scarcely establish the optimum conditions of aeration even for carrot tissue, but they do show that, whilst the three treatments may have given 'similar' results, there were differences, and not inconsiderable ones, between them. There is nothing to indicate that Stiles and Skelding eliminated the source of these vagaries. Referring to the conditions laid down by Steward (see above) for potato discs, Stiles and Skelding remark: 'Steward used bottles of large capacity (2 litres) filled with solution. . . . It is open to question whether the oxygen concentration at the surface of the discs is higher, or even as high, as in a system where there is in the bottle[1] a reserve of air[2] which is brought into contact at intervals with the outside air, at any rate where the experiment is of short duration, as in the case of Asprey's and where the connexion with the outside air is made at fairly frequent intervals and where the system[3] is kept continuously shaken.' This criticism is based on a misquotation, for Steward used bottles of *4 litres* capacity *half-filled* with solution, thus providing a

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[1] Volume = 420 ml. in case of Stiles and Skelding.
[2] Volume not greater than 220 ml.
[3] 200 ml. solution plus 60 discs (2 cm. in diam.; 0·1 cm. thick).

reserve air space, if such were necessary with continuous aeration, five times as great, relative to the amount of tissue surface, as in the technique of Stiles and Skelding.

In one experiment Stiles and Skelding seek to prove that potato tissue under their conditions absorbed as much salt as if under the conditions used

FIG. I.
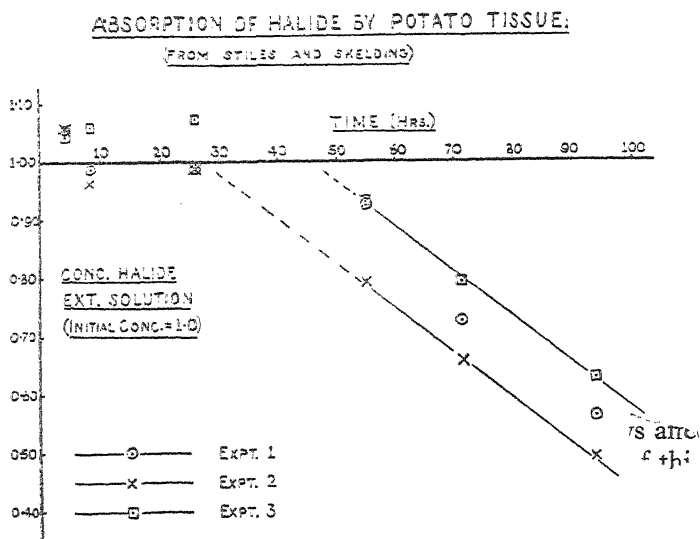
by Steward. To do this they select data from one part of an investigation in this series (IX) to compare with their own, obtained under somewhat similar conditions of temperature, concentration of salt, &c., though it is not entirely clear which of their three methods of aeration they actually used. Stiles and Skelding's point is that their potato tissue absorbed about as much[1] bromide as that used by Steward and Harrison. However, figures from Steward and Harrison cannot be the test of adequate aeration in Stiles and Skelding's experiment. A critical test demands that random-sampled batches of discs from the same stock should be submitted in concurrent experiments to the exact conditions of Steward and Harrison and of Stiles and Skelding.

A significant point, however, is evident in Stiles and Skelding's data from which Fig. 1 was plotted (see Stiles and Skelding's Table II which gives data of three replicated experiments). The figure, which will be referred to later when the form of time/absorption curves is in question, shows clearly that, after absorption had begun,[2] the three triplicate cultures diverged to an extent which suggests that there were still uncontrolled variables at work, and the behaviour in time of the tissue under the prescribed conditions could be fixed only by the two extreme limits shown. It has been the common practice in

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[1] About two-thirds as much on the basis of comparison used by Stiles and Skelding.
[2] Note the evidence here also of a preliminary lag period.

this series to compile data in a single experiment from as many as eight, or even twelve to fourteen, separate cultures and the same degree of divergence between them as shown in Fig. 1 would have obscured many of the conclusions which it has been possible to draw. Stiles and Skelding claim that their aeration system is entirely adequate; but these results hardly support this.

Restating the 'factors affecting salt absorption' Stiles and Skelding cite 'the space relations of the tissue'. The authority for this is the old and meagre data of Ruhland with the confirmation of which Steward, Wright, and Berry are credited. These workers (III) who did not ignore the work of Ruhland, investigated quantitatively the surface phenomena in respiration and salt uptake over a wide range of specified surfaces and disc thicknesses. By methods described (IV) the data were analysed and interpreted and the new conclusions then drawn were consistent with the known behaviour of the cells of potato tissue, and they remain fundamental to the use of such material in physiological work. A later use (IX) of similar methods in the investigation of the absorption of both an anion (Br) and a cation (Rb) again emphasized the great activity in absorption of a shallow layer of cells at the surface of the discs. Stiles and Skelding do not appreciate the essentials of the work which they cite when they speak (l.c., p. 357) of 'dead and dying cells in the neighbourhood of the cut surface' which become 'moribund' and lose their salts, whereas the 'living cells', presumably of the whole disc, slowly absorb. Such a picture of the conditions in a potato disc is erroneous and stands corrected by the data and conclusions of this series which have long been on record. The standpoint of Stiles and Skelding, which neglects the changes in the cells near the surface which culminate in cell-division, overlooks one of the most instructive approaches to the work on storage tissues. New data on these lines will be given in this paper, and it will again be evident that the ability to accumulate salt is closely linked with the ability of cells near the surface to grow and divide.

The analysis of the external solution only, as practised by Stiles and Skelding, has the disadvantage that to make the absorption measurable as a change of concentration the proportion of solution to tissue must be small. This aggravates the problem of total supply and the effect which changing concentration[1] may have upon the rate of salt intake. Stiles and Skelding, like Stiles and Kidd in 1919, ignore the ions initially present in the tissue and also the fate of metabolizable ions (NO_3', PO_4''') after they have been absorbed, and they measure the absorption in units (the fraction of the total salt originally supplied to the tissue in the external solution) which do not

[1] Earlier in this series (V) it was shown that a tenfold external increase of concentration tended to double the quantity of salt absorbed by a standard amount of tissue under standard conditions, and that the effect of the change in concentration which occurred during uptake under these s. ndard conditions was not great compared with the effect of other variables under investigation. Stiles and Skelding remark that in their solutions, none of which was less than $0\cdot001$M, 'the external concentration of the solution may have fallen considerably—often to half, or less, of its original value . . . by the end of the experiment. Usually after four days from the beginning, practically the whole of the ions may have been absorbed from the solution.'

reveal any physico-chemical exchange of ions or disappearance of ions in metabolism.

In this series, on the contrary, notwithstanding the relatively large volume of salt solution, it was considered essential to analyse both sap and external solution and, on occasion, their electrical conductivity. The analysis of tissue fluids was necessary to ascertain the state of the absorbed salt in the cells, a necessity partly due to the adsorption hypothesis for which Stiles was originally responsible. To-day, knowing that the salts exist in true solution in the cells, analysis of the tissue still remains essential to appreciate the behaviour of the various ions and tissue components which play a part in the absorption process. From the recent work of Hoagland and Broyer (1936) and a group of four papers on the biochemistry of salt uptake (Steward et al., 1940) it is clear that full understanding of the problem demands that the behaviour of many solutes, organic and inorganic, electrolytes and non-electrolytes, shall be severally measured. It is, therefore, the more surprising that Stiles and Skelding again confine attention only to the ions of the external solution and seem to deprecate the value of analyses of the tissue (l.c., 1940, p. 337).

Stiles and Kidd referred to what they called 'exosmosis' of ions which was deduced from conductivity data, and Stiles later (1927) wrote of such 'exosmosis' followed by a certain degree of uptake. In 1940 Stiles and Skelding recognized potassium polarographically amongst the ions lost by the tissue early in its contact with the external solutions. A detailed description of the behaviour of the principal inorganic ions during entry of a potassium salt was given early (1932) in this series (I) using the standard methods of chemical analysis. In the present paper the effect of the storage time and temperature of the tubers upon the ability of the cells to retain their salts against distilled water will be described.

Stiles and Skelding now favour the use of the polarograph, which does not distinguish between Cl and Br or between K and other alkali metals, or ammonium and organic bases. This technique, tried by one of us, seemed to lack the necessary degree of specificity and was also subject to interference by solutes other than salts. In our view the standard methods of chemical analysis are the most reliable means yet available for determining salt uptake and they should be supplemented only by methods which are specific.

2. Time and Temperature Effects on Salt Uptake by Potato Tissue

The effect of temperature.

Stiles and Skelding question references made earlier in this series to the effect of both time and temperature on uptake, two variables with which this paper will be directly concerned.

First consider the reference made to temperature effects. It will be recalled that a preliminary experiment (I) revealed the general form of the relationship between temperature and absorption of bromide, although the author recognized the complex interrelationships which were so affected, and

deprecated any deep analysis of the results (I, p. 34). It was noted that the temperature coefficient of absorption was high, as in other cases on record (Hoagland et al., 1926), but the practice of calculating temperature coefficients [Q_{10}], the magnitude of which was so clearly determined by the temperature interval selected, was discouraged. It was also regarded as of some significance that the temperature/absorption relationship showed that at low temperatures (approx. 3°–6° C.), which are associated with other special effects on the physiology of potato tissue, accumulation[1] on the whole-disc basis was not attained.

In other words, it was then foreshadowed that temperature had two distinct effects. The further investigation of the effects of temperature on salt uptake by potato tissue was not in fact neglected. Investigation showed that the preliminary results had given a true picture, extended by further and more precise experiments, and they were, therefore, allowed to stand, unsupported, until that fuller discussion of the effects of temperature on potato discs could be made, including the effect of temperature upon various physiological processes with which salt uptake is interrelated.

Stiles and Skelding (l.c., p. 334) quote from Steward that 'it is only between 14° C. and 20° C. that a temperature coefficient of this order[2] is obtained', and they remark 'Steward is in error in supposing that it is possible to calculate temperature coefficients from single determinations in this way. To express the effect of temperature on salt absorption as a coefficient it is necessary to determine the course of absorption at each temperature and compare the rates of intake at the same stage of intake at the different temperatures.' Doubtless Stiles and Skelding had in mind that in the work of Stiles and Jörgensen (1915) to which Steward (I, 1932) referred, data were given on the course of the process measured with time. Stiles and Jörgensen, however, gave no proof that they really measured *absorption* of H̄ ions, for potato tissue contains buffer substances (see Steward and Preston, 1940) which, when they leach into surrounding fluids, control its [H̄], decreasing the acidity of acid solutions in a manner which, on such evidence alone, might otherwise be ascribed to absorption.

In point of fact Steward did not recommend the use of temperature coefficients calculated for 10° C. intervals; he referred only to the order of magnitude of the temperature effect, and claimed only to indicate the general form of the temperature/absorption relation for potato discs and bromide ions. The data to be given in this paper show that this relationship was correctly described; indeed, data which do yield time/absorption curves at the different temperatures yield a curve almost identical with the earlier one (cf. Figs. 3 and 4 and Fig. 1 of I) when the constants of rate are plotted against temperature.

The effect of time.

The effect of time is of particular interest in shedding light upon the nature

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[1] See section 3 of this paper.   [2] Order of 2·0.

of the absorption process. The earlier work of Stiles and Kidd led to the frequent interpretation of absorption as an equilibrium process, and the behaviour with time, i.e. the course of the absorption, as the progressive attainment of equilibrium conditions. The results of this series were not consistent with such an interpretation and the course of the absorption from dilute solution by potato discs provided but one of many reasons for dismissing that preoccupation with equilibrium criteria which had been a barrier to a correct appreciation of the nature of salt uptake by cells in general and storage tissue in particular.

Whereas in the work of Stiles and Kidd 'equilibrium' was apparently attained after periods of the order of 50 hours, the rate of bromide absorption by potato discs from dilute solution and under the conditions described by Steward continues unabated for a long period, although the steady rate of uptake is not established till after the elapse of a preliminary period to which reference will later be made. This maintained absorption, proceeding for so long a time, must be associated with the progressive metabolic processes which occur in a tissue no longer quiescent but which, despite external signs, exhibits a recrudescence of vital activity which must be identified as growth. The more intimate details of the metabolic processes of such tissue have been investigated and are described in a series of papers on the biochemistry of salt absorption (Steward et al., 1940, et seq.).

Even if attention is confined to the bromide ion and conditions of controlled aeration it is true that different absorbing systems yield time curves which reflect their peculiar characteristics. As shown by Prévot and Steward (1936) several such curves (l.c., p. 525) become explicable in terms of the previous nutrition of the tissue (i.e. whether previously rich or poor in salt) and its capacity for further growth. Potato tissue lacks the brief, but very rapid, uptake of both anion and cation characteristic of the 'low-salt' cells and, on the contrary, even requires a period to adjust to the conditions which permit rapid uptake of the bromide ion—a period during which metabolic processes gather momentum. Once begun, bromide uptake occurs from dilute solutions of alkali salts at an approximately constant rate and the anion is accompanied by cation. During the initial lag period, however, some ions (e.g. Rb) may be absorbed by the discs, but the behaviour with respect to specific surface, oxygen tension, and time is such that this appears to be different in kind from the subsequent protracted absorption being not so dependent upon the metabolic processes of the cells. The behaviour in time could be accounted for (IX) on the assumption that, from dilute solution, the course of the absorption of salt was linear with time after the elapse of a preliminary period during which cation (Rb), but not anion (Br), was absorbed by a purely equilibrium mechanism. The data to be given now are also consistent with this interpretation, with the added observation that the duration of the lag period increases at lower temperatures. The limitations which must eventually restrict the further progress of absorption from dilute solution by potato have not yet concerned us—beyond the recognition that they are long deferred and are not

merely to be interpreted in terms of simple physico-chemical equilibrium as earlier supposed.

Stiles and Skelding return to the course of the absorption of salts by storage tissue and their experiments refer mainly to tissue from the carrot root (an organ which presents more anatomical problems than the potato tuber) and various salts of the alkali metals. They also comment upon the interpretation of time curves in the uptake of alkali bromides by storage tissue described elsewhere in this series.

In the range of data graphed by Stiles and Skelding (comprising the results of absorption experiments with different salts at various concentrations) it is not easy to discern any very consistent relation between absorption and time beyond that the uptake continued to occur throughout the period of observation. Within series in which different concentrations of the same salt were investigated some curves cross and even recross and, at comparable concentrations, different experiments with either the same or different salts yielded curves which are sometimes dissimilar. Stiles and Skelding state that what might appear to be but casual fluctuations in the rate of uptake represent real properties of the cells and, moreover, that they can discern in one set of previously published data for potato, evidence consistent with the interpretation they now apply to carrot. For these reasons the position needs to be re-examined.

The variation between replicate experiments with potato by the method of Stiles and Skelding has been referred to (see Fig. 1), whereas we know that the behaviour of this uniform tissue can be accurately replicated. Admittedly the bulk of Stiles and Skelding's time curves refer to carrot, but this would hardly reduce the difficulty of duplication. Before considering Stiles and Skelding's interpretation of time curves it may be asked whether, with this degree of spread between parallel cultures, some of the irregularities in their published time-absorption curves represent anything more than casual variations which, whether due to the factors referred to above or to others, must be eliminated before the true relation of absorption to time can be discerned.

Whereas from Stiles and Kidd it seemed that equilibrium was attained after periods of the order of 50 hours, the position according to Stiles and Skelding is as follows. At first the storage tissue loses potassium and the more so in distilled water or very dilute salts than in stronger potassium salt solutions. Loss of anion in this period is much less evident than the loss of cation (K). 'Sooner, or later, the exosmosis of potassium gives place to absorption . . . and for a time, usually about two or three days, the rate of absorption, rapid at first, gradually declines. This course does not, however, continue and gives place to a phase during which the rate of absorption increases.' Thus Stiles and Skelding identify three stages in the course of salt uptake by carrot tissue: phase I, characterized by the loss of solutes already present in the cell (particularly K), phase II characterized by absorption of salts at a rate which declines with time, and phase III characterized by a renewed period of uptake at an increased rate.

Glancing at Stiles and Skelding's published time curves it will be clear that the limits of these three phases are not clearly defined. The loss of potassium which is the characteristic of phase I is a usual concomitant of the storage tissue technique (see I, Table 8). Under similar conditions it occurs more with potato than with carrot, but should not be regarded as peculiar to cations (K) because the accompanying anions may be organic (I and Steward, Stout, and Preston, p. 412). In this series the initial loss of potassium has been regarded as a property of cells which have not yet attained that degree of metabolic activity necessary to absorb rapidly, but which eventually do so the more readily if they are well aerated, &c. In other words, the loss of potassium is properly not a feature of the absorbing system, but is attributable to the fact that at the start of experiments the metabolically inactive potato tissue, unless exposed to a pretreatment in aerated water, has not yet acquired either its full capacity to retain or to absorb solutes.

According to Stiles and Skelding phases I and II merge, and the implication is that a declining rate of uptake in phase II is again the behaviour which Stiles and Kidd observed, though it is now said to be followed by phase III. The important point of interpretation is clearly the transition between phase II and phase III (in both of which absorption of anion and cation occurs), a transition which is supposed to occur after two to three days, and must, therefore, be attributable to some development in the tissue at this time. If Stiles and Skelding had not approached their interpretation from the standpoint of Stiles and Kidd, that there was a period of declining rate of uptake as of the approach of equilibrium, it is doubtful whether they would have seen any evidence of transition from one phase of uptake to another at two to three days. In our view there is no evidence for this in the published curves, whereas it is evident that, even in relatively strong solutions, uptake occurs progressively between about 10 and 80 to 90 hours at rates which, if the supply is still adequate, show but little sign of decline, and recognizing the variability in replicate cultures, the time curves might, with few exceptions, not unreasonably be represented as the straight lines of nearest fit whose slope would then measure the rate of absorption. If small differences in the rate of salt uptake occurring after about two to three days are to be regarded as real, it seems that an estimate of their degree of significance is required. It is reiterated, therefore, that even in Stiles and Skelding's own case the proper interpretation seems to be that, although the form of the time curves may indicate that other complications are encountered in the *early* stages of absorption during the first few hours after contact with the salt solution, the essential feature is that after these have elapsed uptake continues until at least 80 to 90 hours without noticeable signs of abatement. In this the results on carrot and potato tissue are in essential agreement.

Stiles and Skelding remark that the 'published contributions to our knowledge of the course of salt absorption by storage tissue made by Steward and his collaborators consist of two or three experiments in which the absorption of bromide from potassium bromide was followed'. The point is that the

results published were from experiments made two years apart with different varieties and in widely separated laboratories. The data were almost superimposable for identical external conditions and this seemed an adequate indication that the behaviour in time as described was not an isolated or casual result: sufficiently so that the publication of confirmatory data from similar experiments seemed hardly necessary. Incidental to another investigation the behaviour in time of the uptake of rubidium bromide by potato tissue was investigated and these results were published, not because the behaviour in time of bromide absorption seemed in any doubt, but because essential differences in the absorption of rubidium and of bromide—in which the behaviour in time formed but a part of a more comprehensive analysis—shed light on the nature of the absorption processes.

Stiles and Skelding seem to imply that the contributions in question were scanty and therefore to be accepted with reservation. It should be made clear that in so far as the effects of time are concerned the new data are consistent with the old and are in accord with the remaining body of evidence, published and unpublished, from work on potato tissue by the methods of this series. The time and temperature data published here were available long before (they date from 1930–1) the paper of Steward and Harrison was written. No apology is needed for adhering to a form of analysis similar to that used by Steward and Harrison. That the form of the time/absorption curves is exactly that which has been described on two previous occasions adequately indicates that it represents more than a casual result, and reveals an essential feature of the behaviour of potato tissue under the conditions described. In possession of so concordant a body of data few could remain in doubt as to the general form of the absorption/time curves.

Stiles and Skelding refer to the paper by Steward and Harrison (1939). The conclusions which Steward and Harrison drew came from a consideration of the effects of three different variables on uptake of the ions rubidium and bromide by potato discs, and a crucial group of experiments, carefully planned to reveal their interrelations, was published. These experiments were not designed without knowledge gained from preliminary work—the details of which were not given. Stiles and Skelding isolate from their context the data which refer to the effects of time, reinterpret them according to their own views, and make some errors of fact when referring to the data.

It was emphasized (IX) that rubidium alone may be absorbed from a solution of its salt by storage tissue in a manner which bears a distinctive relationship to the variables, time, specific surface, and oxygen supply. The limit of the uptake which occurs by this means is rapidly reached, i.e. in relatively few hours. In two places (p. 331 and p. 355) Stiles and Skelding erroneously state that Steward and Harrison 'claim' that rubidium uptake alone continues for 'two days' or for 'a day or two'. An essential part of the analysis was that this preliminary uptake of rubidium, which it was shown could occur in dead cells, was rapidly completed and was over before the onset of simultaneous uptake of rubidium and bromide—a process which did

not begin till some hours after the discs were put in the experimental conditions. In a given case the behaviour in time was summarized by the following two equations:

$$[\text{Rb}] = 5 \cdot 0 + 0 \cdot 173(t - 9 \cdot 0)$$
$$[\text{Br}] = 0 \cdot 173(t - 9 \cdot 0),$$

where 5·0 mg. equivalents per l. of water represents the basal rapid uptake of rubidium, 9·0 hours represents the period during which simultaneous uptake of rubidium and bromide is slow or absent, and the constant of rate identifies the subsequent approximately steady rate of absorption which obtained under the conditions specified for the remainder of the experimental period. That the duration of the 'lag period' was subject to control was recognized and it was shown that by pretreatment of the discs in aerated distilled water it could be shortened.

Prior to Steward and Harrison's work the time curves in this series were constructed from data on rather large, replicate cultures which were sampled *in toto*, so that each time curve contains data from many parallel cultures with but small variability between them. For reasons incidental to that investigation and as explained in the text, Steward and Harrison necessarily analysed much smaller batches of tissue sub-sampled from fewer replicate cultures—so that the effects of variability (not the errors of single analyses, cf. Stiles and Skelding's comment on p. 355) were necessarily somewhat greater than in the earlier work. Stiles and Skelding in their Fig. 11 use the data which Steward and Harrison represented in their Fig. 4. Steward and Harrison calculated a line of nearest fit, and the special feature of their interpretation was that it was consistent with the whole body of evidence. Stiles and Skelding, omitting the actual points of reference, draw freehand curves, using an arbitrary choice of scale. They not only suggest that these freehand curves more justly interpret the original data, but they attach significance to certain slight irregularities as they have drawn them, seeing in these evidence of some real property of the tissue and of an increased rate of uptake which, occurring after some three days, they believe to be comparable to that which they also maintain occurs in the case of carrot tissue.

The present authors still claim that all the data on the effect of time on the uptake of bromide by potato discs are adequately represented by straight lines and do not indicate the inflexion, after some 40–80 hours, of two merging time curves as Stiles and Skelding hold.

Stiles and Skelding remark that 'the third of the earlier long period experiments is that by Steward and Berry in which the absorption from potassium bromide by artichoke tuber of only the bromide was observed by daily measurements of the absorption over a six-day period'. Stiles and Skelding say that 'there was, however, no indication of an increased rate of absorption at any time, the rate remaining approximately constant'. Stiles and Skelding give an erroneous account of the way in which this investigation was carried

out and they do not quote correctly either the experimental procedure, results, or the conclusions of Steward and Berry (VII).

The respiration of artichoke tissue exhibits a time drift different in kind from the behaviour of potato discs. It was the purpose of the artichoke experiments to ascertain how the tissue, as it passed through this time-respiration drift, varied in its ability to absorb salt. To this end the bromide, chloride, and specific conductivity of expressed sap and external solutions were measured in concurrent experiments in which the respiration of tissue subjected to the following treatments was measured: (1) artichoke discs continuously in unchanged distilled water, (2) artichoke discs continuously in unchanged KBr—initially at 0·00075 M, (3) by using six replicate cultures the absorption per 24 hours from 0·00075 M. KBr was measured at six different portions along the time/respiration drift. Prior to the twenty-four hours contact with the salt the tissue was in distilled water. It will be seen that neither on p. 355, nor in their Table 1 (p. 332), do Stiles and Skelding indicate correctly the conditions under which this experiment was carried out.

The results of Steward and Berry's investigation on artichoke discs were briefly these: (1) The conductivity data were consistent with the interpretation that, after the initial period of absorption during which complications are encountered, the bromide ion was accompanied by its cation potassium in approximately equivalent amounts.[1] (2) The uptake of bromide in the first period of contact with the external solution, a period during which respiration was high, remained lower than this might have led one to suppose because other and more mobile ions derived from the tissue itself re-entered during this period. (3) After the completion of the behaviour indicated in (2) the falling rate of respiration[2] was consistently accompanied by a falling *rate* of salt absorption indicated alike by bromide analyses and by conductivity data. The 'approximately constant rate' to which Stiles and Skelding refer is not shown by the figures given by Steward and Berry (VII; p. 110, Figs. 3, 4).

(4) A transient effect of KBr on the respiration of artichoke discs was also noted.

The case of the artichoke tissue thus reveals a type of time/absorption behaviour which is distinct from that observed in potato. Care has been taken to emphasize that the form of the time/absorption curves is determined by recognizable properties of the cells, notably their ability to grow and their previous mineral nutrition, and to these should be added the tendency to fix ions, of one sign or the other, by purely chemical means analogous to base exchange in soils. A discussion on these lines by Prévot and Steward (1936), to which Stiles and Skelding do not refer, shows how in the behaviour of different systems the operation of these factors on salt uptake may be discerned and various forms of time/absorption curves thus explained.

[1] Stiles and Skelding say that the bromide ion *alone* was observed.

[2] Experiments have been made to ascertain the reason for the falling respiration with time and the concomitant decrease of bromide uptake. The case of artichoke tissue is beyond the scope of this paper, but it may be said that the results are so far consistent with the view developed later in this paper that the limiting factors are related to nitrogen metabolism.

The interaction of time and temperature

Almost without exception all investigations, between o° C. and 25° C., show that the higher temperatures are the most conducive to salt absorption. Unanimity is not, however, complete, for Petrie (1927) claimed that although the uptake of anions is increased at the higher temperatures, that of the cations
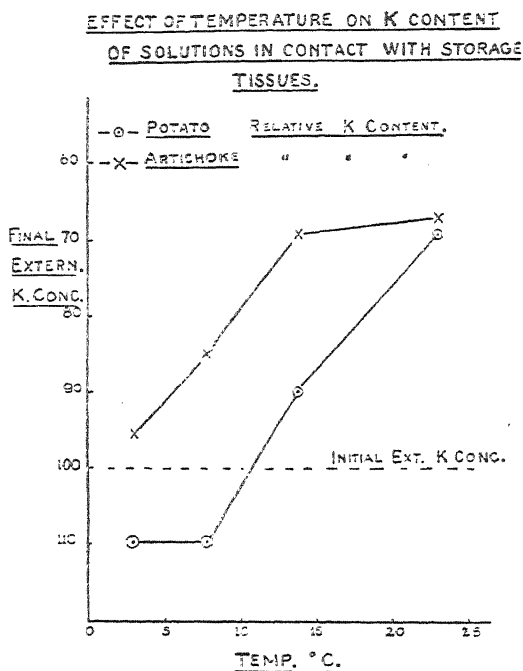
FIG. 2.

is decreased and that the salt relations of the tissue are regulated by the condition that, as affected by temperature, the external ionic product remains constant. Although this idea was suggested for storage tissue it does not apply even to these tissues under conditions which are conducive to salt absorption, for then the effect of temperature on anion and cation is similar in kind. Though the following treatment refers mainly to the bromide ion it is emphasized here that in the case of potato discs, artichoke discs, and potato roots the effect of temperature on cation uptake is similar in kind to that on anion absorption, namely greater absorption occurs at the higher temperatures, and the external ionic products, far from remaining constant, show a *total* decrease at the higher temperatures.

After arbitrarily selected time intervals the effect of temperature on the potassium content of solutions (initially o·75 mg. equiv. per litre) in contact with artichoke and potato discs is given in Fig. 2. Potato discs in aerated solutions at temperatures of o° C. to 5° C. actually lose some of the potassium they originally contained and this loss exceeds the slight uptake of bromide at

these temperatures. The data of Hoagland and Broyer (1936) on barley roots show that the uptake of *both* positive and negative ions is increased by temperature. In fact the advantages to be derived from the modern technique of heating the solutions in large-scale water cultures emphasize the same point.

Undoubtedly the effects of time and temperature interact. As an example the data of Hoagland and Broyer (1936) will suffice. These show that excised barley roots, in which the rate of salt uptake is markedly affected by time, also respond in different degrees to temperature according as this effect is investigated earlier (e.g. 10 hours) or later (e.g. 24 hours) in the response of the roots to time. Similarly, the effect of temperature on the relative uptake of anion and cation may not be constant but reflects changes in the system in which time plays an essential role. To elucidate fully such complex relationships or to draw generalizations demands much investigation. The case of potato discs is, however, somewhat simpler.

For comparatively long periods the behaviour of potato discs in time is relatively simple. After a brief lag period the uptake of bromide commences, and proceeds at a steady rate during a prolonged period, in the course of which the mean respiration rate remains almost constant. From dilute solutions of potassium bromide the tissue absorbs anion and cation in equivalent amounts. Loss of potassium due to the effects of low temperatures or of the experimental technique itself on the inherent ability of the tissue to retain its salts does not, of course, apply to bromide which is not initially present. The uptake of bromide, therefore, yields data which reflect the effect of temperature on the absorption unaffected by these complications.

The standard technique of this series (II) was used and the apparatus for the control of temperature was that described by Steward, 1933. The potassium bromide solutions employed were initially 0·00075 equiv. per litre and, to enable the behaviour in time at each temperature to be determined, discs were subsampled from the cultures at various times. Duplicate cultures were used at each temperature (i.e. 23·2°; 13·7°; 7·7°; 3° C.) and on one of each pair (which was sampled *in toto* for bromide determination) the respiration rate was measured.

The data lead to Fig. 3, shown in isometric projection to reveal simultaneously the effects of temperature and time on the bromide concentration in the cell sap, as well as the time which elapses (lag period) between placing the discs in the experimental solutions and the beginning of bromide uptake at each temperature.

From the figure it will be evident that both the duration of the lag period and the subsequent rate of bromide uptake are conditioned by temperature, and that the sigmoid absorption/temperature curve which appeared in the preliminary experiments described in 1932 and was called in question by Stiles and Skelding in 1940 (p. 334) again appears as a constant feature of the results. This sigmoid curve clearly expresses a general property of actively absorbing well-aerated systems since it is discerned also in the results of Hoagland and Broyer (1936) on roots.

Clearly, below 5° C. absorption is drastically curtailed by temperature.

Between 5° C. and about 15° C. the greater absorption due to increase of temperature is most striking, but above this temperature other factors enter which tend to restrict absorption. These results have a possible bearing on
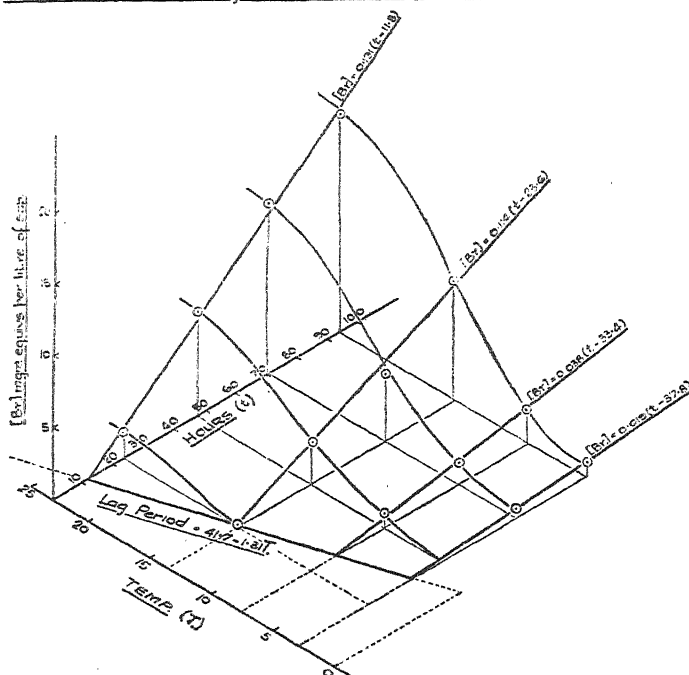
FIG. 3.

modern techniques which involve growing plants in solutions the temperature of which is separately controlled.

At each temperature the relation between absorption and time is clearly given by a straight line (see discussion on p. 230 et seq.), and the rate of bromide uptake is best identified by the constant of slope which reveals, in its variation with temperature, the effect of this variable on absorption, free from confusion with effects due to time (see Fig. 4). The calculated lines of nearest fit have equations which, expressed in the form[1] $[Br] = a(t-b)$ used earlier (IX), are as follows:

$$
\begin{aligned}
\text{Temp.} & \\
23 \cdot 2° \text{ C.} \quad & [Br] = 0 \cdot 191(t-11 \cdot 8) \\
13 \cdot 7° \text{ C.} \quad & [Br] = 0 \cdot 114(t-23 \cdot 6) \\
7 \cdot 7° \text{ C.} \quad & [Br] = 0 \cdot 038(t-33 \cdot 4) \\
3° \text{ C.} \quad & [Br] = 0 \cdot 019(t-37 \cdot 8)
\end{aligned}
$$

[1] In this form the bromide concentration [Br] is expressed in mgm. equivalents per litre of sap, *a* is the constant of rate, *t* the duration of the absorption period in hours, and *b* the lag period in hours. Experiments with sodium bromide solutions and similar temperature conditions gave results comparable to Fig. 3 and are, therefore, not included.

The increase with lower temperatures of the time elapsing before bromide uptake is expressed by the relationship: lag period $= 41 \cdot 7 - 1 \cdot 31 T$. This is interesting because it indicates that, if those other factors which at higher temperatures restrict the rate of bromide absorption did not also affect the lag period, absorption should begin immediately at about $32°$ C., whereas at $0°$ C.,

EFFECT OF TEMPERATURE ON RATE OF BROMIDE UPTAKE AND DURATION OF LAG PERIOD IN POTATO DISCS IN 0·75 mgm.EQUIV. KBr.
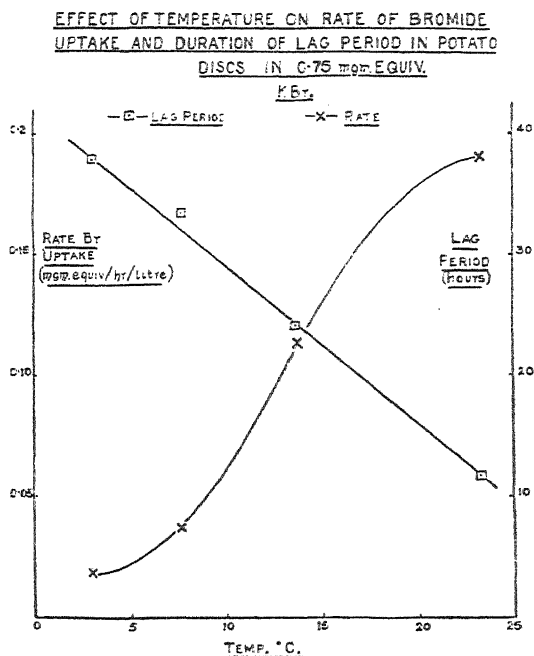
FIG. 4.

although the tissue might be able to absorb bromide after some 40 hours, the rate of uptake would be vanishingly small (see Fig. 4). Between about $7°$ and $17°$ C. the tissue shows its greatest response to temperature, and in this range the temperature coefficient may be very high (Q_{10} of the order of 4 to 5 in the case quoted), but the value of the temperature coefficient is so plainly dependent upon the range chosen[1] that this form of expression has no special significance. It will be clear that the conclusions and comments made in 1932 from the preliminary experiment are fully confirmed.

Effect of temperature on respiration.

The relative effects of temperature on respiration and bromide uptake are of interest. Examples of the general parallelism between the effects of specified variables on these two properties of the discs have been noted earlier, and again there is evidence of such a relationship. For convenience the data are compared on a relative scale of units in which the bromide concentration in

[1] See page 239 for suggestion that the surface effects confuse the calculation of temperature coefficients and when this confusion is eliminated they approach more normal values.

The distribution of absorbed bromide in the discs is likewise affected by temperature. Stress has been laid upon the fact that at 23° C. the absorbed bromide is confined to cells of which the respiration is much increased above the basal value common to the cells far from the disc surface, and that there is a steep gradation from the outermost cells which absorb most bromide to
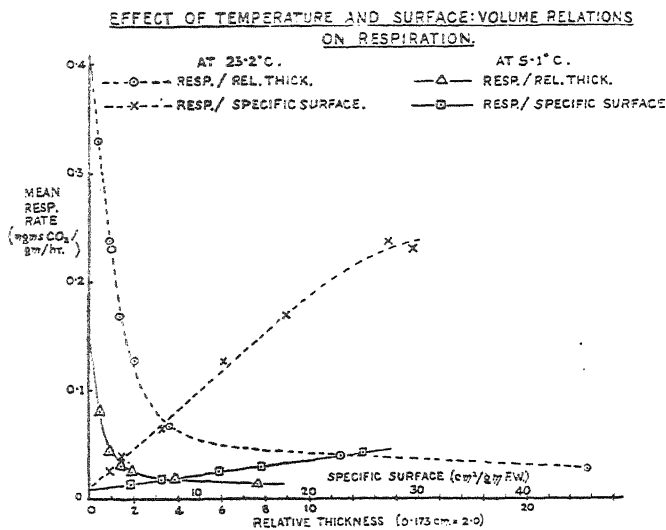
FIG. 6.

those in which there is little or none. These phenomena were investigated at 2·8° C., 7·8° C., 13·8° C., and 23·2° C., and the full analysis demanded by the procedure described (IV) was carried out. The results will not be given in full. The conclusion is that the depth of tissue ($\bar{z}$) which contains bromide decreases at the lower temperatures; the values of $\bar{z}$ which measure the depth to which bromide penetrated, are 0·015, 0·031, 0·040 cm. at 7·8°, 13·8°, and 23·2° C. respectively. A complication, not evident at the higher temperatures except in very strong salt solutions, which operates increasingly at the lowest temperatures, is that the greatest total concentrations in whole discs are encountered not in the very thinnest (0·037 cm.) but in those somewhat thicker (0·073 cm.). The explanation of this is deferred and it was neglected in the calculations referred to.

Effects due to protracted washing.

The effect of protracted washing in running tap water on the subsequent intake of salts by storage tissue presents, in a special form, the interaction of time and temperature on the behaviour of discs of storage tissue.

Running tap water may exert its influence, if any, upon the tissue by virtue of its temperature, salt content, or oxygen concentration. The effect of any such factors which tend to cause loss of solutes from the vacuole will be

accentuated by the constant removal of the leached components. Since the beginning of these investigations on storage tissue knowledge that the duration of the washing period was a variable which affected subsequent intake under the standard conditions of experiment was met by the arbitrary standardization of the duration of washing during any given series of experiments.

At the temperature of running tap water (below 10° C. in the experiment of Table II) potato tissue, but *not artichoke*, loses potassium, and no doubt other solutes, to aerated solutions (Fig. 2). Such loss of solute should engender a deficit of total solute, somewhat analogous to that which may be produced during the growth of plants under low salt supply, and when the conditions for salt intake eventually return, such a deficit should permit a greater intake of salt in a given time the greater the length of the previous washing. As shown by bromide uptake this effect occurs, and the vicarious salt intake thus caused by long washing is not accompanied by a *proportional* change in the respiration of the tissue (Table II).

If unduly prolonged, protracted washing causes the property of salt accumulation to disappear, and this occurs whilst the tissue retains its ability to respire. Although the effects of long washing in tap water on the biochemistry of the tissue (see section 4) and on its ability to grow (see section 3) would repay examination, there can be little doubt that the effects of washing on subsequent intake are mainly explicable in terms of the leaching of the solutes (organic and inorganic) from potato tissue, which this treatment entails at the comparatively low temperatures of running tap water. The relative immunity from the effects due to washing, of artichoke tissue, which does not lose potassium to aerated solutions at 2° C., supports this view with which Asprey's data (1937) are not inconsistent.

TABLE II

The Effect of Washing in Tap Water on Subsequent Bromide Intake by Potato Discs during 48 hours at 23° C. from KBr Solution (0·00075 mg. equiv. per litre)

Duration of washing (hrs.)	Resp. rate (mg. CO_2 per gm. hr.)	Bromide conc. in sap (mg. equiv. per litre)
1	0·187	5·81
25	0·191	13·04
50	0·214	18·94
553	0·210	0·73

The effect of storage temperature on salt accumulation.

The effects of temperature thus far described refer mainly to the way in which this variable affects the *rate* at which cells exercise their inherent capacity for salt uptake. In potato tissue, however, the previous storage time and temperature of the tubers determines whether the cells are *capable* of absorption even under conditions of temperature, &c., which are normally

conducive to salt accumulation (thin discs in dilute, aerated solution at 23° C.). It will be shown that the previous storage time and temperature affects those metabolic processes in the cells which are most closely associated with salt accumulation and, therefore, the further investigation of this aspect of their response to temperature affords the opportunity for some essential attributes of cells capable of salt accumulation to be defined.

The problem was first approached in the following manner. One of us (F. C. S.) in 1933 had access to a supply of tubers previously stored for many months at various temperatures (0°; 2°; 4·5° C.) as well as control tissue stored at 12°C. At about 2° C. the tissue showed the high sugar content and high rate of respiration commonly found in such material. The expectation was that such tissue would prove to be even more metabolically active than that from normally stored material and in consequence absorb more salt. The converse was the case. It was shown that discs from this material failed to accumulate bromide, even under the most favourable conditions, either at 2° C. or 23° C. In fact, they lost the solutes they previously contained and, after a preliminary swelling[1] (by about 3 mm. on a normal 17-mm. diam.), the tissue eventually lost much of its turgor. Other tests of viability, however (e.g. protoplasmic streaming), showed that the cells treated at 2° C. were still alive.[2] Here then was a case in which living potato discs, rich in sugar and active in respiration, yet lacked the capacity to accumulate bromide. Investigation showed that it could be correlated with the behaviour of slices which, when exposed to moist air at room temperatures, did not heal or form a meristem.

3. The Effect of Low Temperature Storage on Meristematic Activity of the Cells of the Potato Tuber

Attention will be confined to results obtained on tubers after long storage at 2° C. At lower temperatures (0° C.) irreversible physiological breakdown occurs, and at higher temperatures (4·5° C.) the symptoms which are of interest are not so well developed.

Tubers after long storage at 2° C. were transferred to room temperature (16–18° C.). At various subsequent periods (26, 51, 77 days) slices were cut from selected tubers and exposed to moist air.[3] Simultaneously, control tubers, drawn directly from cold storage (2° C.) were similarly treated. The progress of the wound-healing reaction (suberization, phellogen formation, protoplasmic streaming, &c.), if any, was investigated on sections cut at right angles to the upper surface which had been in contact with air.

The cells of tubers stored at 2° C. were entirely free of starch grains and they did not regain their starch during the periods of observation of the cut slices. In moist air the slices became very turgid and both at 2° C. and at

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[1] The swelling itself is not the cause of failure to accumulate. Experiments were made in which the swelling was restricted by use of salts, glycerine, or sugar solutions without affecting this behaviour.

[2] For brief reference to this work see Steward, 1935.

[3] On moist filter-paper, in trays, covered with glass plates.

room temperature active protoplasmic streaming could be seen along the tenuous cytoplasmic strands. As normally stored (e.g. at 11° C.) the cells of the potato tuber do not exhibit streaming, although this does develop as cells become more active and starch-free during the development of a phellogen. The low-temperature storage treatment, however, completely inhibited this

THE EFFECT OF LONG STORAGE AT 2°C. ON HEALING AT A
CUT SURFACE IN MOIST AIR AND ROOM TEMP.

UPPER SURFACE, SLICES FROM COLD STORED TUBERS, 14 DAYS
AT ROOM TEMP.
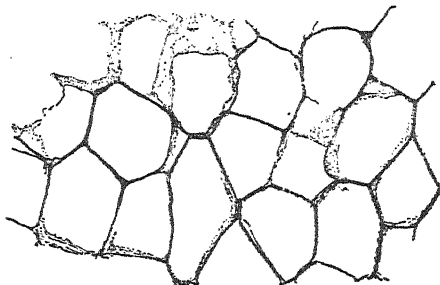
UPPER SURFACE, SLICES FROM COLD STORED TUBERS 51 DAYS
AT ROOM TEMP. HEALED 14 DAYS.
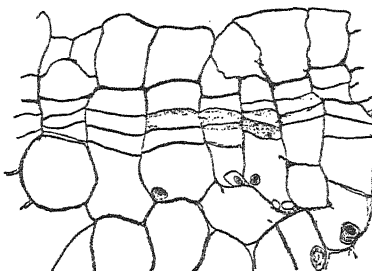
FIG. 7.

normal response in the cells near the surface of the slice which should form a meristem when exposed to air. However, after tubers have had a period of recovery at room temperature from the effects of prolonged storage at 2° C. their cells regained the ability to heal in moist air. The longer the period of exposure of the tubers to room temperature the more readily did slices cut from them heal and form a meristem. Fig. 7 shows the behaviour of slices after 14 days in contact with moist air. When the slices were cut from cold-stored tubers, although the old cell-walls suberized, no cambium initials were laid down and no cell-divisions of any kind occurred. Similar slices, cut from tubers which had recovered during 51 days from the cold-storage treatment, responded almost normally though somewhat more slowly. After 51 days at room temperature the slices developed 2 to 3 new cell-walls in 14 days by divisions in otherwise mature parenchyma; scattered starch grains occurred deeper in the slice than the phellogen. After 77 days at room temperature the

rate of healing was greater (4 to 5 new walls in 7 days) and the parenchyma within also contained abundant starch grains. During recovery the buds also grew, though the growth at the high temperatures of the room was anomalous, producing stolons and new tubers directly.

The effect of cold storage on the ability of the parenchyma cells to grow and divide and form a meristem is therefore reversible. Meristematic activity is only suppressed by *prolonged* storage at low temperatures and it is regained only after a comparatively long treatment at room temperature. In both the inactivation and the eventual recovery time as well as temperature is an essential factor. In neither case is the response one which could be explained simply in terms of the expected effect of temperature on the *rate* of a physiological process. Like the associated response of carbohydrate metabolism and of respiration the effect of temperature on healing must be attributed to the highly specific action on the living system of a strictly limited range of low temperatures. Whatever its explanation, the low temperature response does show how closely integrated are certain apparently unconnected properties of the living cells.

Berry and Steward (VI) attached significance to the capacity of cells to grow as an indication of their ability to accumulate salt. Therefore, by investigating the development in time of the low temperature response, the failure of such tissue to accumulate salts could be linked more closely with the physiological and biochemical characteristics of the cells.

The formation of a phellogen in moist air is visible evidence of growth of which protein synthesis may be regarded as a quantitative measure. New experiments were, therefore, so planned as to indicate how storage time and temperature affected not only the composition (both with reference to the carbohydrate and nitrogen fractions) of discs cut from the tubers, but also the influence which they have upon the changes which ensue when the discs are subjected to conditions conducive to salt uptake for a specified period of time (48 hours). During this test period the gain or loss of salt by the cells was investigated either by direct analysis (e.g. Br) or indirectly by the measurement of electrical conductivity.

4. THE EFFECT OF STORAGE TIME AND TEMPERATURE UPON THE COMPOSITION AND SUBSEQUENT BEHAVIOUR OF POTATO DISCS AT 23° C. UNDER CONDITIONS CONDUCIVE TO SALT ACCUMULATION

The stock used was of the variety King Edward VII. One portion was stored at 2° C.,[1] the other (referred to as the normal storage material) was stored at 11° C. The latter served as control material to the former, but, since the experiment was prolonged to 16 months the tubers finally sprouted and the data of the cold-stored material stand alone in the later periods. At stated intervals tubers were selected at random from each storage condition and discs were cut by usual methods (II). The long washing customary in these

[1] By arrangement with the Union Cold Storage Co., London.

experiments was reduced to a brief rinse. Analyses of the carbohydrate (reducing sugars and disaccharides) and nitrogen (alcohol soluble, protein, and amide) fractions were made on such discs before and after they were exposed for 48 hours at 23° C. to 0·001 M KBr and the standard conditions of the salt accumulation experiments. The biochemical data were calculated on the standard *initial* fresh weight[1] of the discs to eliminate effects due merely to differential swelling and water intake. During the period of contact with the
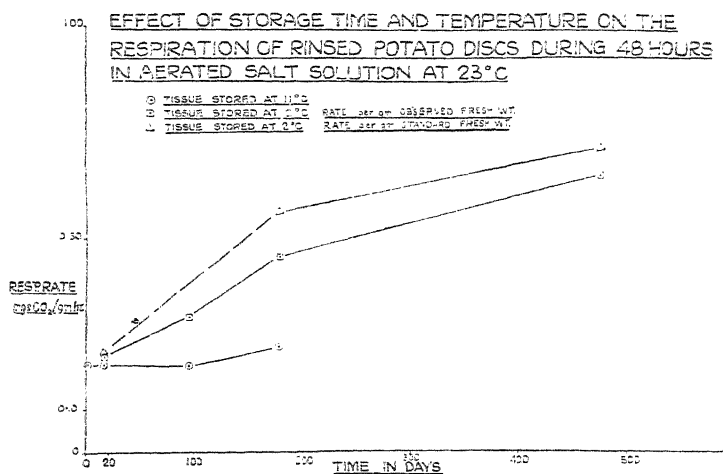
FIG. 8.

salt solution the respiration was measured and also the change in the conductivity of the external solution.

The biochemical background for the determination of the nitrogen fractions is to be found in the work of Steward and Preston (1940). Methods and the *raison d'être* for the otherwise arbitrary choice of the fractions selected for analysis follow from the results of the earlier work.

The effect of storage time and temperature on the respiration and salt relations of potato discs at 23° C.

The relevant data are shown graphically in Figs. 8, 9, and 10. The usual respiratory performance of standard discs of normally stored potato tubers was consistently reproduced at a mean rate of approximately 0·20 mg. CO_2 per gm. hour. Even at the extreme end of the rest period, when buds were beginning to develop, the central tissue from large tubers respired at a rate only slightly in excess of that amount (Fig. 8). On the contrary, storage at 2° C. caused the respiration of cut discs to increase progressively with storage time—the increase being perceptible before 20 days of such storage and after

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[1] Weight of rinsed discs of standard size and normal sugar content.

2oo days the respiration was approximately double that of discs from normal tubers.

Figs. 9 and 1o confirm the effect of long storage at 2° C. on salt accumula-

EFFECT OF STORAGE TIME AND TEMPERATURE ON THE ACCUMULATION OF BROMIDE BY RINSED POTATO DISCS IN AERATED 0·00IM POTASSIUM BROMIDE AT 23°C. DURING 48 HOURS.

FIG. 9.

EFFECT OF STORAGE TIME AND TEMPERATURE ON LOSS OF SALTS FROM RINSED POTATO DISCS TO AERATED SALT SOLUTION· AT 23°C.

FIG. 1o.
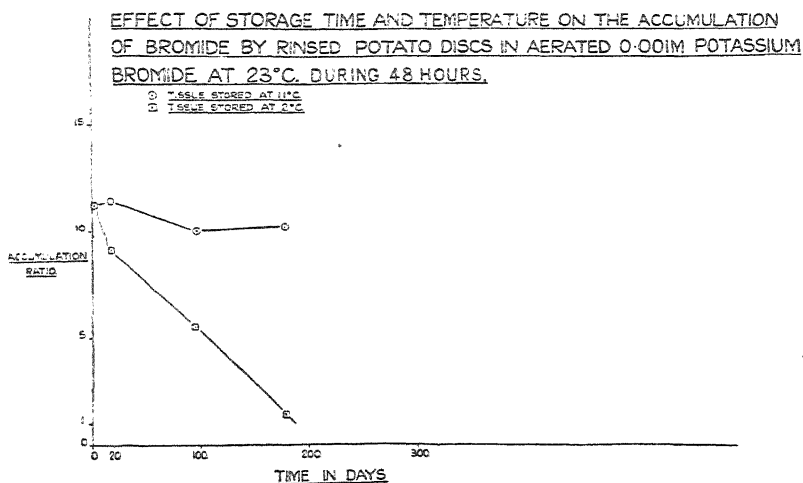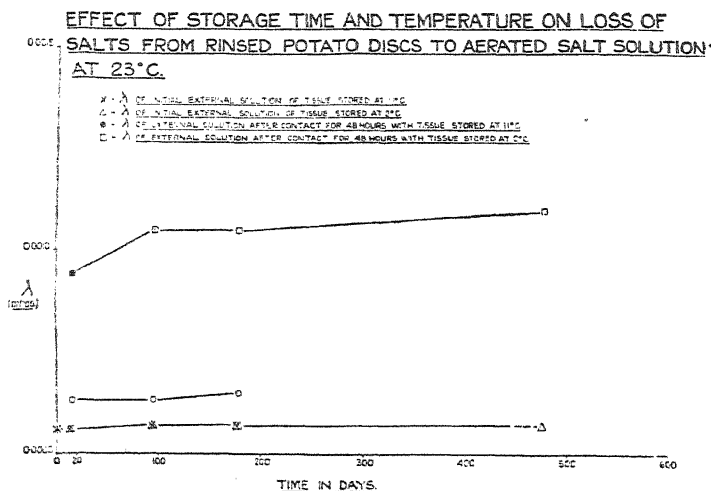
tion previously referred to, and they also reveal its time relations. Even towards the end of the normal rest period standard discs under standard conditions retained their ability to accumulate bromide (accumulation ratio somewhat greater than 1o; see Fig. 9); any decrease in accumulation with

protracted storage at 11° C. was slight and in a direction opposed to the small concomitant increase of respiration. From the start of storage at 2° C. the bromide absorbed by cut discs, during the subsequent standard absorption period of 48 hours, decreased rapidly and, after about 200 days, the tissue had lost the capacity to accumulate bromide. This progressive decline in the capacity to accumulate was accompanied by an actual increase in respiratory activity. Clearly carbon dioxide production and capacity to accumulate salts are not directly related. Only when, in its rate of production, carbon dioxide reflects the rate of other vital processes which are more intimately concerned with salt uptake does the parallelism between respiration and salt uptake obtain (see also Steward, 1935, 1937).

Fig. 9, however, does not reveal the effect of storage time and temperature on the ability of the cells to retain the salts already present in their vacuoles before this treatment. Even potato discs cut from normally stored tubers lose ions to the external solution (I) in the early stages of these experiments, and though some of these ions are subsequently reabsorbed a total decrease in electrical conductivity is not observed immediately the absorption of bromide begins (e.g. at about 12 hours, see Fig. 3). Fig. 10 shows that the conductivity of the salt solution, even after 48 hours' contact with discs from tubers stored at 11° C., was increased somewhat and by an amount not inconsistent with these known features of the behaviour of potato tissue. It was not so, however, in the case of the discs from cold-stored material, for this released salts which increased the conductivity of the external solution (2 litres to 30 gm. of tissue) by a relatively large amount. It is noteworthy that by 100 days, when the tissue still accumulated much bromide, this effect had already reached its maximum (see also Fig. 9).[1]

The consequences of storage at low temperatures are, therefore, not merely metabolic in character, not merely concerned with the absorption of ions hitherto absent from the cells, but they also include effects which would by some be attributed to changes in the permeability properties of the cell-membranes. Protoplasmic streaming, which can be more easily observed in this starch-free cold-stored material than in normal potato tissue, is a convenient proof of its viability, which the behaviour described might otherwise call in question, although, as emphasized later (see p. 250), the tissue did not attain equality of concentration of its diffusible constituents with the external solution. The point stressed is that even the *ability to retain solutes (ions and non-electrolytes alike)* is dependent, not merely upon the passive properties of a membrane, but upon a system with definable metabolic characteristics. This was indeed foreshadowed by the observation that at low oxygen tensions potato cells lose chloride and potassium, and that an unexpectedly high rate of metabolism is necessary to retain these solutes (V). As will appear later, the

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[1] The consistency with which the standard conditions used in these experiments reproduce the salt uptake of potato tissue is evident by comparing Figs. 3 and 9. From Fig. 3, although this experiment was done with a different variety, the accumulation ratio at 48 hours was 9·45.

property which seems above all to determine the ability of the tissue to absorb and retain solutes is protein synthesis.

Effect of storage time and temperature on the metabolites of potato tissue and their subsequent behaviour under conditions conducive to salt accumulation

Particular attention was paid in this investigation to those processes which
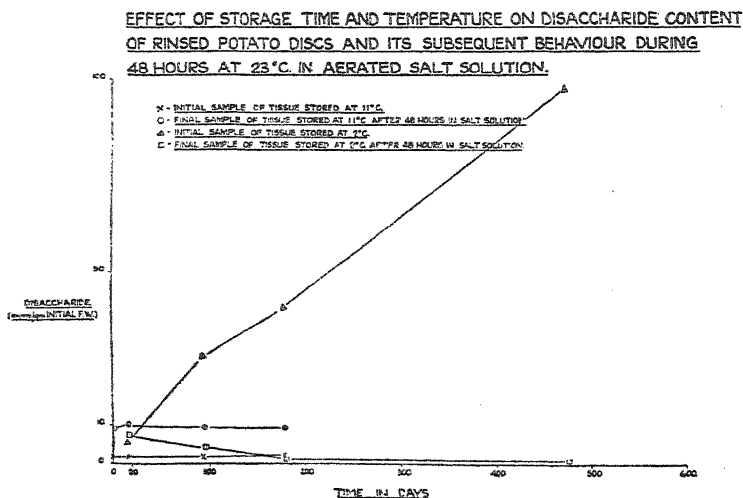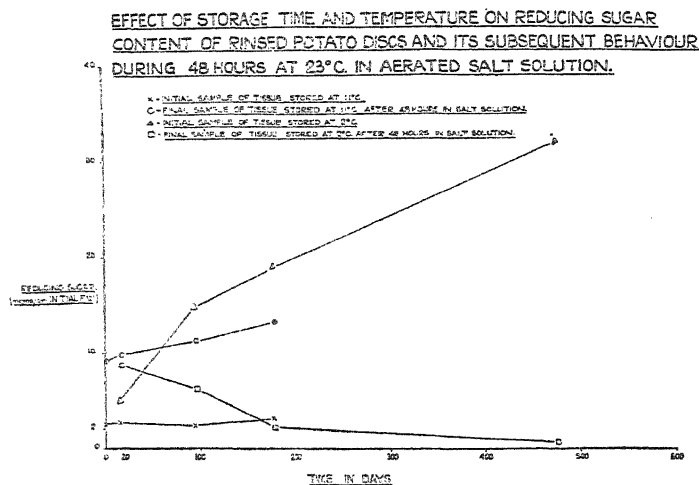
FIG. 11.

from other work (Steward and Preston, 1940) were known to be the salient features of the metabolism of the discs.

Carbohydrates. Tissue from tubers stored normally, cut into thin discs and exposed to aerated solutions, undergoes starch hydrolysis which is localized in the surface cells (III), is conditioned as to its amount by the concentration of

dissolved oxygen (Steward, Stout, and Preston, 1940) but is affected but little by the presence of dilute salt solutions (Steward and Preston, 1940). The sugar content of normally stored potato tubers remains at a low level and the sugar is composed of both reducing sugars and disaccharides, though somewhat more of the former. The curves of Fig. 11 show that throughout the 200 days of storage at 11° C. the content of reducing sugars and disaccharides in the tubers, as drawn from storage, changed but little about the mean values for each component (2·65 mg. reducing sugar, 1·90 mg. disaccharide per gm. fresh weight), but, when this tissue, in the form of thin standard discs, was exposed to aerated 0·001 M KBr solution, both reducing sugar and disaccharide fractions were much increased, reaching a level of the order of 10·0 mg. per gm. of initial fresh weight. After longer storage at 11° C. the total starch hydrolysis (starch equivalent of the gain of total sugar plus the starch equivalent of the respired carbon dioxide) was progressively increased and the sugar residue became, in larger measure, composed of reducing sugar rather than disaccharide. The drift in time of respiration at 23° C. of discs from normally stored tubers (11° C.) is more nearly parallel to the concentration of the reducing sugar than the disaccharide fraction.

With long storage at 2° C. the sugar content (Fig. 11, initial samples) of the tubers increased progressively from 15 days, when a significant gain had occurred (reducing sugar 5·10, disaccharide 5·50 mg. per gm. of initial fresh weight), until the end of the period of observation (475 days), by which time a very great increase (reducing sugar 32·10 mg., disaccharides 98·40 mg. per gm. initial fresh weight) had taken place at the expense of the starch reserves. This is an example of the familiar effect (first attributed to Muller Thurgau, 1882, later investigated by Hopkins, 1924, Appleman and Smith, 1936, Barker, 1936, and others) which is usually associated with the unexpectedly high rate of respiration which ensues when many starch-containing tissues are returned to higher temperatures after storage at low temperature. The general similarity in the effect of low temperature on respiration and sugar content was noted by Hopkins, but a causal connexion between them apparently only operated up to a certain limiting concentration beyond which further increase in sugar even depressed respiration.

Hopkins suggested, though without any data in support, that following the old views of Spoehr and McGee an increase in amino-acid content of tubers stored at low temperature might have contributed to their respiratory activity —a suggestion which is of interest in relation to results yet to be described (p. 253).[1]

As is usually the case, Fig. 11 shows that, after low temperature storage, the relative increase of the disaccharide fraction is greater than that of the reducing sugars (cf. the increase of sugar in cut discs of normal tubers) but, as shown, both metabolites attain very high levels. It is usually thought that in potato tubers sweetened by low temperatures the principal sugar is sucrose, and

[1] Comparing Figs. 13 and 8 it is clear that the increase of respiration begins *before* the increase of soluble nitrogen compounds becomes very apparent.

Barker (1936) concludes that this is the sugar which provides the immediate respiratory substrate (said to be γ-fructose) and the one which regulates the rate of respiration of 'cold-stored' *whole* potato tubers. The results show that as affected by low-temperature storage—in marked contrast to the effects of salts—respiration and sugar content (both reducing sugars and disaccharide) of the cut discs do vary concomitantly.

When cold-stored (2° C.) tubers were cut into thin discs and submitted for 48 hours to the conditions of salt absorption experiments the sugar content (reducing and disaccharide) fell, and to levels below those maintained under similar conditions by tissue from normal-stored (11° C.) tubers. Even the whole of the respired carbon dioxide would not account for this change and there is no doubt that the sugars, like the salts (Fig. 10), diffused out into the external solution. It will be noted that this effect did not become evident till after some 20 days of storage; it increased progressively with the length of the previous storage period, and it was not due to the attainment of equality of the sugar concentrations inside and outside the cells (a condition which demands final internal concentrations of approximately 1/70 of the initial).[1]

The interpretation is that after prolonged storage at 2° C. the cells, which normally can retain their organic as well as inorganic solutes against a steep diffusion gradient, exhibit this property in a diminished degree according to the length of the storage treatment. The loss of salts was conspicuous after 15 days of storage, the loss of sugars only became conspicuous somewhat later. After 80 to 90 days the leaching of salts (which are limited in amount in the tissue) had almost reached its maximum, but the leaching of sugar still increased (reflecting its production from starch) to 200 days and thence more slowly to the end of the period of observation.

Protein nitrogen fraction. Potato tissue is rich in alcohol-soluble nitrogen, which is mainly amino-acid nitrogen (Steward and Preston, 1940), and, though the proportion of alcohol-soluble to alcohol-insoluble nitrogen (which in potato tissue is the protein N) varies somewhat with the stock, the variety, and the conditions of storage, it is often of the order of 2:1. At the beginning of the period of observation for the material used in this work, and stored at 11° C., this ratio was nearer 1:1.

During a further 200 days of storage at 11° C. protein breakdown did not occur and the tissue retained its normal ability to accumulate bromide ion, to retain the bulk of the solutes in its vacuoles, and even to increase its protein content by synthesis during 48 hours in 0·001 M KBr at 23° C. (see Figs. 12 and 13). Analysis of discs cut from tubers which had been stored at 2° C. showed that the protein content of the tissue fell progressively, bearing an approximately linear relationship to storage time. This indicates that protein breakdown, as well as starch hydrolysis, occurred during the storage at 2° C. On transfer to the conditions conducive to salt uptake the tissue only regained its normal protein level for these conditions after 15 days of storage treatment at 2° C. After longer periods of storage the protein level declined progressively

[1] Only at the very end of storage (475 days) and relative to disaccharide only could this be true.

in a manner which indicated (Fig. 12) that after some *85 days of storage (at 2° C.) the tissue when transferred to dilute, aerated salt solutions at 23° C. could no longer synthesize protein, did not maintain its existing protein content, and*
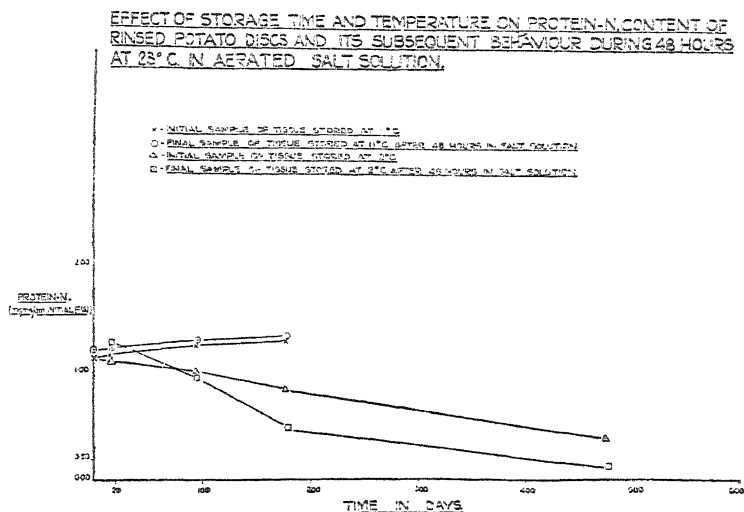
Fig. 12.
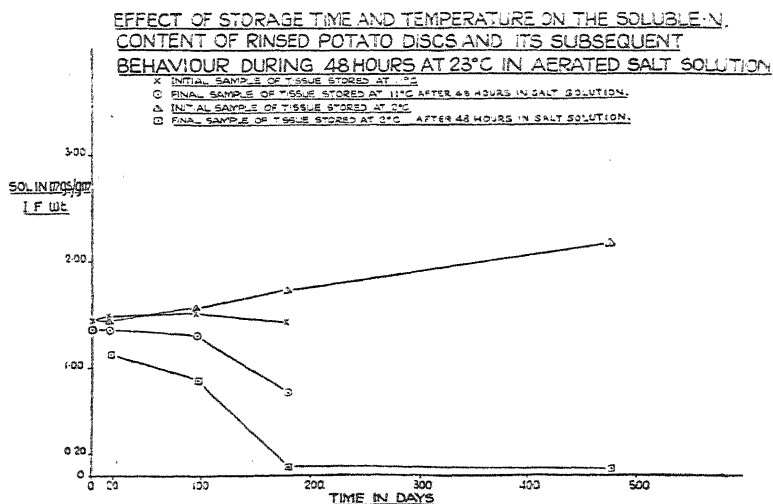
Fig. 13.

responded to these conditions, normally conducive to synthesis, by an accelerated breakdown of protein.

The data for alcohol-soluble nitrogen content produce the complementary picture (Fig. 13). Alcohol-soluble nitrogen, which is almost constant under storage at 11° C., increased progressively with storage time at 2° C. (from

1·44 mg./gm. F.W. at 15 days to 2·18 mg./gm. F.W. at 475 days). This corresponded to the concomitant decrease in protein nitrogen by breakdown, already referred to (from 1·05 mg. protein N/gm. F.W. at 15 days to 0·39 mg. at 475 days). During the 48-hour period in which the discs were exposed to aerated salt solution at 23° C., the soluble nitrogen content decreased, but only after long storage (greater than 100 days) at 11° C. was there a marked loss of soluble nitrogen compounds to the external solution. From discs cut from cold (2° C.) stored tubers an extensive loss of soluble nitrogen, not due to protein synthesis, occurred (at 23° C.) by diffusion into the external solution, and this, which became evident even after 15 days of the low-storage treatment, continually increased in amount with the length of the previous low-temperature treatment. Clearly soluble-nitrogen compounds, like sugars and electrolytes, leached out of the cells which, previously submitted to cold-storage treatment, were subsequently placed in aerated salt solutions at 23° C.

Hence, the impressive feature of the response of potato tissue at 2° C. is not merely the effect which this treatment has upon its *total composition* but rather that it progressively reduces its *capacity to synthesize protein even under conditions normally conducive to this process.* Prior to some 80 days of storage at 2° C. the tissue still retained a sufficient residual capacity for synthesis to maintain its initial protein content but, subsequently, accelerated breakdown obscured any slight synthesis which may have occurred. In this connexion great interest attaches to the observation that after *prolonged storage at 2° C. the superficial browning of the discs, commonly observed in aerated solutions, did not occur.* This property, due to the action of the polyphenol oxidase of the potato, has been linked with the deamination of amino-acids, and thence with protein synthesis, respiration, and salt uptake (Steward, and Preston, 1940, and 1941).

Soluble nitrogen fraction. Until about 100 days storage at 2° C. and 11° C. does not produce much change in the various soluble nitrogen constituents. Free ammonia is only present in traces in normal potato tubers and this is also true of those after cold storage at 2° C., though the amount did increase progressively from 0·06 mg. NH_3 nitrogen at the beginning of the period to 0·14 mg. at 477 days. True amino-N,[1] which is mainly amino-acid nitrogen, constitutes the largest single fraction of the soluble nitrogen of potato tubers and, for the case in question, there was little difference in the level of this fraction at the temperatures of 2° and 11° C. during the first 80 days or so. This was also the case for the heat-stable and heat-unstable amide fractions (see Fig. 14). *However, approaching 80 days of storage a fundamental change in the nitrogen metabolism of the tissue occurred, for it lost its ability, when in the presence of free oxygen, to synthesize protein in excess of that necessary to replace the protein constantly being broken down in the cells.* Subsequently, not only did the total protein content decrease with storage time but it could not be restored again at 23° C. in aerated salt solutions (see p. 251

[1] Not to be confused with heat-unstable amides which also contribute to the Van Slyke amino-N fractions.

and Fig. 12). From this time the curves of the soluble nitrogen fractions at 2° C. and 11° C. storage deviate, indicating that the former condition caused the increase of amino-N and of the heat-unstable amide whereas the stable amide fraction declined.

Amino-acid nitrogen appears as a product of protein breakdown. The
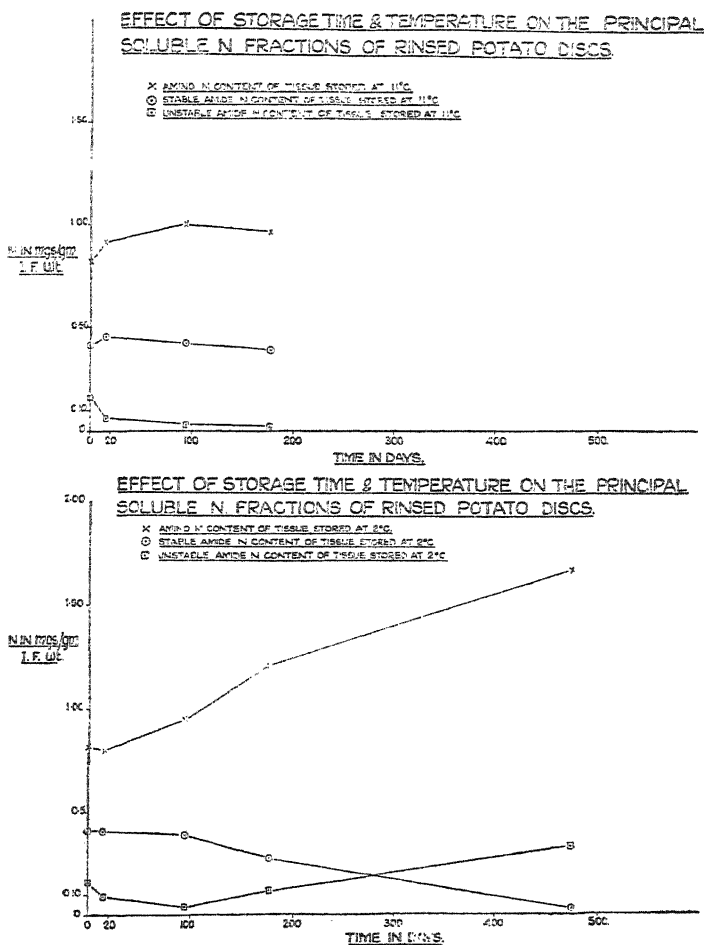
FIG. 14.

nitrogen for protein synthesis is normally drawn from this fraction (Steward and Preston, 1940), and in material capable of synthesis the *oxidase system is active*; it has been suggested that oxidase deaminates amino-acid, yielding ammonia. Other evidence suggests that the heat-unstable amide fraction may be an intermediary, via ammonia, between the main reserve of amino-nitrogen and protein, and the conditions which make for a relatively low rate of synthesis also permit ths unstable amide to accumulate. In cells able to

grow, synthesis of protein, carbohydrate-consumption, and a high respiration rate tend to be associated, and cases have been noted in potato discs where relatively high sugar content is associated with a relatively high unstable amide content—so much so as to suggest a possible relationship between these two fractions.

The data of Fig. 14 are clearly consistent with this picture. It has been stated that after long storage at $2°$ C. the activity of the oxidase disappears. The free ammonia accumulation which occurred arose not from deaminated amino-acids but from amide by hydrolysis, and without the necessary intervention of the oxidase system. The decrease in the stable amide fraction was, however, greater than the small increase of free ammonia and the excess, in a tissue rich in sugar, was converted to the heat-unstable amide fraction which, since protein synthesis was suppressed, accumulated.

5. GENERAL CONCLUSIONS

The outstanding feature of the results is that prolonged storage of tubers at $2°$ C. inactivates at one and the same time the ability of the cells to grow, as shown by healing in moist air, by their ability to synthesize protein, and by the metabolic properties such as phenolase activity, deamination of amino-acids, &c., which are linked with it. The bulk of the respiration which the low-temperature stored tissues exhibit is independent of that component of the total respiration which in cells able to grow seems to be linked with protein synthesis and not controlled by the sugar concentration of the cells (Steward and Preston, 1940, 1941). Barker shows that the respiration of whole potato tubers under low-temperature storage is conditioned by their sucrose concentration. The significant point, however, is that this enhanced metabolism as shown by carbon-dioxide production does not produce a system active in salt uptake or able to retain its solutes, organic and inorganic, against aerated distilled water. Lacking the capacity to synthesize protein and all those other metabolic processes linked with it, which in their summation confer the ability of the cells to grow and divide, the cells lose their ability not only to accumulate bromide but also to maintain existing concentration gradients of both electrolytes and non-electrolytes.

Protein synthesis, therefore, emerges as the cardinal feature of cells which are capable of ion accumulation and which retain what used to be called their semi-permeable properties—properties hitherto ascribed to the passive behaviour of a membrane but which now, it seems, demand definite metabolic requirements. Following the classical experiments of De Vries and Pfeffer, which culminated in the osmotic view of plant cells, there have been various experimental re-examinations of that ability of the cells to retain the contents of their vacuoles under long contact with distilled water which constitutes the evidence for their so-called semi-permeable properties. A summary of the experiments of Wachter, Puriewitsch, Grünfeld, and others was given by Steward (1928). Looking again at this old problem, in which the literature admittedly is not unambiguous, from the standpoint of this new investigation

it is suggestive that the tissues which provide the classical evidence of prolonged ability to retain organic and inorganic solutes, e.g. beetroot (especially when sterile and aerated), contain living cells which are still able to grow—whereas this is not true of those which lose their contents to aerated distilled water (e.g. onion scales, endosperm tissues, &c.).

In the case of potato tissue there is clear evidence that the ability of the cells to absorb and accumulate salt, as well as to retain their electrolytes and non-electrolytes for long periods against distilled water, is asssociated with their ability to synthesize protein, to grow and divide. Further investigation is clearly desirable, but there are good grounds for the belief that this relationship may be widespread in living plant cells. Functions usually supposed to reside exclusively in a plasma membrane, albeit one so intimately a part of the living system that its very efficiency has been a test of viability, now appear to demand more of the cell merely than that it should be alive—it must be capable of anabolic processes of which protein synthesis is a convenient index. Only in so far as carbon-dioxide production is linked with these reactions does its rate provide an index of the ability of the cells to accumulate and retain solutes; carbon-dioxide production *per se* is not alone effective.

The effects of temperature on potato discs are, therefore, twofold. The first operates directly upon the rate of metabolic processes and of salt uptake of tissue which, being drawn from normal storage at temperatures well above those at which starch hydrolysis occurs in whole tubers, retains its normal capacity for growth as revealed by cell-divisions in moist air. Here the main effect of temperature is on the rate of processes which, though they may not be in active operation in the dormant tuber, are rapidly set in train in thin discs exposed to dilute aerated salt solutions at 23° C. The duration of the 'lag period' is a measure of the time taken for this adjustment. Temperature affects both the duration of the lag period and also the steady rates which are ultimately established. The facts have been shown in Figs. 3 and 4. No doubt the effect of previous long storage time at 2° C. on bromide uptake at 23° C. (Fig. 9) might be described in terms of effects both on the duration of the lag period and on the subsequent rate of the ion intake which occurs. One could visualize the lower total bromide uptake, e.g. after 100 days, as due to either a longer lag period or a slower rate of intake after bromide absorption eventually begins or even to both of these possible results of long storage at 2° C.

The evidence is, however, that there is here a second type of temperature response which, developing at low temperatures during a long time, expresses itself by depressing certain metabolic processes and salt intake, even when the tissue is transferred to those higher temperatures and to conditions in which it is normally active. Barker (1936) postulated such a depressant for respiration, because he recognized that the sugar-rich cells of 'sweetened' potatoes respired at normal temperatures at rates less than their high sugar content would otherwise lead one to expect. The present evidence is that after long storage at low temperatures the tissue responds differently to conditions which are normally favourable for growth and metabolism. The reason is that the

storage treatment inactivates, though reversibly, a part of the vital machinery. Low temperature storage inactivates protein synthesis which, linked to salt uptake and at least a part of the aerobic respiration of normal potato discs, constitutes both an essential feature of growing cells and of the 'dynamic machinery' whereby salt is accumulated and solutes are retained in the vacuole.

SUMMARY

Part 1 contains a rediscussion of the technique of storage tissue experiments with special reference to problems which are raised by a paper of Stiles and Skelding (1940). These problems concern effects of aeration, time, concentration and supply of solute, and the surface-volume relations of discs of tissue on the uptake of ions, and also the bearing of the methods of analysis on the experimental investigation of the problem. Conclusions previously reached in this series are reiterated.

Part 2. The form of the published time/absorption curves is discussed.

An experimental re-examination of the effects of time and temperature on the absorption and accumulation of bromide by potato discs is described. The lag period (previously noted I, IX) before the uptake of bromide by potato discs occurs is increased at lower temperatures, and when absorption eventually begins it is at a slower rate at the lower temperatures.

The time/absorption behaviour can be represented by linear equations (as in IX) in which the effect of temperature on bromide uptake is shown by the constant of rate. The value of this quantity bears to temperature a sigmoid relationship similar to that previously described for the effect of temperature on the accumulation ratio. The interrelated effects of time and temperature on the duration of the lag period and on the course of bromide uptake have been summarized by a figure which is shown in isometric projection and by equations which have been derived.

The immediate effects of temperature on respiration and bromide uptake are compared. Temperature conditions which make for high respiration rates in discs previously stored at 11° C. also produce greater bromide and potassium absorption although no direct stoichiometrical relationship between respiration and ion intake can be discerned. At low temperatures bromide uptake ceases whereas carbon dioxide is still evolved, and, at the higher temperatures (e.g. 23° C.), bromide absorption is evidently restricted in a manner which does not apply to respiration.

Temperature also affects the surface phenomena in respiration and bromide uptake. The great effect of temperature on respiration of potato discs in salt solutions is mainly an effect on the so-called 'surface-respiration' (III, IV), which is the amount by which the respiration of a thin shell of tissue is increased beyond the rate of cells deep-seated in the discs or in the tuber from which they were cut. The 'surface-respiration' is not only increased by temperature in its limiting intensity at the outer surface of the discs but also in its extent within the discs as measured by the depth at which it ceases and cells respire at the rate typical of the tissue in bulk ('bulk-respiration').

Calculations show these dimensions for discs in dilute salt solution at $5 \cdot 1°$ C. and they are compared with those which obtain at $23 \cdot 2°$ C.

Similar factors also influence bromide uptake which, at $5 \cdot 1°$ C. as well as $23 \cdot 2°$ C., is confined to cells with a metabolic rate (respiration) above that of cells deep-seated in the disc.

The effect of temperature on the intensity and distribution of surface phenomena complicates the calculation of temperature coefficients and accounts for the unexpectedly high values which these may attain.

Part 3. After prolonged storage of potato tubers at low temperatures $(2°$ C.) discs cut from them have unexpected properties. Though they have a high respiration rate, and initially, a high osmotic pressure which causes them to swell and absorb water, they yet fail to accumulate salts and even lose the electrolytes previously stored in their vacuoles. In the latter respect they resemble discs from normal tubers immediately after they are immersed in solutions at $23°$ C. and before the necessary degree of metabolic activity has developed, or discs which are in contact with solutions in equilibrium with oxygen at a lower partial pressure than that of air (V). Slices cut from such tubers will not form a meristem in contact with moist air—either at the temperature of storage ($2°$ C.) or at room temperature. By their swelling in moist air, protoplasmic streaming, and the eventual recovery of their normal properties their viability was established, and it was shown that the effect described constitutes another specific and hitherto unrecognized effect of long storage at low temperatures.

Recovery at room temperature of the ability of the tissues to heal in air, like the previous loss of this property, involves time as an essential factor with temperature—both effects appear only after a long period. Anatomical data which are recorded illustrate these points.

The formation of a meristem in moist air is visible evidence that the cells can grow. The suggestions derived from this work were given a metabolic basis in section 4. From the investigation of nitrogen metabolism it is shown that after about 85 days of storage at $2°$ C. a crucial change in the metabolism of potato discs occurs; this period is probably the minimum at which the anatomical effects described could be observed.

Part 4. The effect of storage time and temperature on the composition and subsequent behaviour of discs cut from potato tubers has been investigated. Stocks of tubers were stored at $2°$ C. and $11°$ C. At intervals discs were cut from random sampled tubers and their composition with respect to carbohydrates and various nitrogen fractions determined before and after a period of 48 hours, under standard conditions normally conducive to salt accumulation, during which their respiration and bromide uptake was measured.

Prolonged storage at $2°$ C. progressively decreased the subsequent accumulation of bromide during 48 hours at $23°$ C. After about 80 days' storage accumulation no longer occurred—in other words, the tissue no longer responded to the conditions conducive to salt uptake by exhibiting the metabolism with which salt uptake is linked. Though starch hydrolysis

occurred and respiration was raised by the storage treatment far above that normally shown by potato discs under these conditions, this alone did not lead to salt uptake. On the contrary the cells lost first (e.g. after 15 days) their ability to retain salts (shown by conductivity measurements on the external solution) and later (e.g. after about 40 days) their ability to retain sugars and (after 100 days) their soluble nitrogen compounds.

The normally stored (11° C.) tubers, when cut into discs, did not merely maintain, they even increased during 48 hours at 23° C. their protein content by synthesis. Low-temperature storage (2° C.) was accompanied by slow protein breakdown and, after about 85 days of storage, the protein content of the tubers was no longer increased, or even maintained, by synthesis at 23° C., but at this higher temperature accelerated breakdown occurred. The fact that such tissue did not conserve its solutes against distilled water suggests that for semi-permeability (i.e. the maintenance by cells of existing solute concentrations against distilled water) there are special metabolic requirements in addition to the presence of membranes in a living protoplast.

The proportions of the principal components (true amino-N, heat-stable and heat-unstable amides) of the total soluble nitrogen were not greatly affected by the low-temperature storage until after such time as the tissue could no longer increase its protein content by synthesis. From this time the amino-N increased progressively with time as protein breakdown occurred. Parallel with the increase of amino-N and sugar the heat-unstable amide-N also increased and—although at a very low level of absolute concentration— the same was true of ammonia-N: the concomitant decrease of stable amide-N suggests that hydrolysis of amide was the source of the ammonia-N.

Normally in potato discs the nitrogen for the bulk of the protein synthesis arises by deamination of amino-acids, and the concurrent activity of the phenolase system (itself determined by similar variables) suggests that the phenolase activates the deamination. The unstable amide fraction, formed apparently from ammonia and sugar and appearing to be an intermediary of synthesis, decreases when synthesis is accentuated and accumulates when it is retarded. After long cold storage the cells of potato tuber lack both phenolase activity and protein synthesis, but during the storage period slow amide hydrolysis released ammonia which, in the sugar-rich tissue, yielded the unstable amide fraction and this, in the absence of synthesis, tended to accumulate.

The low-temperature storage treatment diverts nitrogen metabolism from the sequence amino-acid→ammonia→ 'unstable amide'→protein. In tissue from tubers stored at 11° C. it is the first stages of this sequence of reactions which are especially linked with salt uptake and the aerobic respiration with which it is constantly associated.

A sharp distinction is, therefore, drawn between the *immediate* and the *deferred* effects of temperature on the behaviour of potato discs. The immediate effects illustrate the control of temperature over the rate at which the tissue from normally stored tubers exercises its capacity for salt uptake,

respiration, protein synthesis, &c., in cells which are still able to grow. This brings out again the general parallelism between salt accumulation and metabolic activity. Long storage at low temperature produces, reversibly, in the cells a milieu unfavourable to growth, to protein synthesis, to salt accumulation, and to the retention of solutes in the vacuole; and the chief feature of this *deferred* effect of low-temperature storage on the cells is the inhibition of their capacity to synthesize protein. Ability to synthesize protein thus emerges as that metabolic property of potato discs which, above all others, distinguishes cells which are able to accumulate bromide and to retain solutes in the cell sap.

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The Permeability of the Cellulose Cell Wall

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With two Figures in the Text

1. INTRODUCTION

A CONSIDERABLE amount of work has been done on the permeability of the cell wall and various semipermeable and impermeable walls have been described. Such are the walls of the barley pericarp investigated by Brown (1907, 1909) and other pericarps and testas investigated by Shull (1913) and Rippel (1918). These walls are coated with cutin or impregnated with other substances responsible for the semipermeability. The cell walls of the onion, found by Brooks (1917) to be impermeable to various chlorides, are also markedly cutinized. A review is given by Stiles (1924). The unaltered cellulose wall is generally regarded as highly permeable to solutes. Stiles discusses this question and gives data of the absorption of various substances by cellulose to support his view that 'the cell-wall cannot be dismissed as a factor in permeability'.

Fitting (1915), in work on the permeability of the cells of Rhoea to salts, notes that, in some of his results, the wall as well as the protoplasm may play a part; but he gives no experimental evidence bearing on the point. In a later paper (1917), on isotonic coefficients, he states that the time for maximum contraction of the protoplast in solutions of non-penetrating salts was 15–30 minutes; in sucrose solutions it was 60–120 minutes. He refers this to differences in rates of diffusion of the solutes, and adds that nothing is known as to whether the cell wall may not offer greater resistance to the passage of sucrose than to that of salts.

Höffler (1930) and Huber and Höffler (1930) examined the question incidentally to their work on the permeability of the protoplast to water. Their method was based on the time course of plasmolysis and deplasmolysis. This may be affected not only by the resistance of the protoplast to water, but also by the resistance of the wall to the passage of the solute used for plasmolysis. If, for example, the wall were less permeable to sucrose than to urea, plasmolysis would be slower in the former than in the latter. It was found that mesophyll cells of Maianthemum showed a similar course of plasmolysis in sucrose, urea, and various salts, and the possibility of differential permeability of the wall was rejected. In measuring the rate of deplasmolysis it was desirable

to know the state of the solution between protoplast and wall. In a piece of tissue placed in liquid paraffin, when partially plasmolysed, contraction of the protoplast continued. From this it was argued that very rapid passage of sucrose through the wall had taken place, as the solution between wall and protoplast must have been markedly hypertonic.

The second paper surveys a wide variety of material from all groups of the plant kingdom, in regard to the magnitude and variation of protoplasmic resistance to the passage of water. The possibility that slow rates of plasmolysis might be due to cell-wall resistance was carefully examined. Low wall permeability was found in the leaves of various mosses. It was most clearly demonstrated in glucose solutions in which, for some mosses, shrinkage of the cell as a whole took place and plasmolysis was much delayed. The leaf cells of *Hookeria lucens* were rapidly and uniformly plasmolysed in 0·5 M sodium chloride, while in 0·8 M glucose plasmolysis was very slow and irregular; there was evidently differential permeability of the wall as regards these two solutes. This effect in the mosses is referred to cutinization of the walls. Strugger (1935, Fig. 20) gives an illustration of cells of *Mnium splendens* which clearly show the buckling of the cell walls and the absence of plasmolysis in sucrose solution.

For cells with non-cutinized walls the rates of plasmolysis varied widely. There are plants the cells of which plasmolyse in potassium nitrate in 1 minute and others which require as long as 15 minutes. When the behaviour to solutes of different molecular size was studied, it was found that cells which plasmolyse rapidly plasmolyse more rapidly in malonamide or potassium nitrate than in sucrose. Examples are Vallisneria and Zygnema. Cells which plasmolyse slowly show no time difference between those solutes. Examples are Salvinia and Spirogyra. Where the protoplast is readily permeable to water and plasmolysis is therefore rapid, differential permeability of the wall to solutes becomes evident. Where the protoplast offers high resistance to the passage of water, the wall may be differentially permeable, but this may not become evident. In Bryopsis, Ectocarpus, and other seaweeds the wall is so impermeable to sucrose that buckling of the cell occurs in sucrose solutions.

A further experiment was carried out with cells of the onion scale epiderm. If a strip is plasmolysed and cut across in water, some uninjured protoplasts may be squeezed out and from these the sucrose is immediately washed away. Such protoplasts deplasmolysed more rapidly than those within cell walls, indicating that there is some resistance by the wall to the diffusion of the solute. De Haan (1933), who used the onion epiderm in work on permeability to water, also dealt with this question and concluded that sucrose was washed out of the space between protoplast and wall very rapidly; the wall was therefore highly permeable to sucrose. There is here a difference of opinion; but the general work of Huber and Höffler makes it certain that, in a wide variety of plants, the wall does offer resistance to the passage of solutes and that this resistance is differential. The work was incidental to other problems and no quantitative study was made.

2. PRELIMINARY OBSERVATIONS

The present investigation started from a chance observation during class experiments on suction pressure. Discs of suitable storage tissue immersed in a series of solutions of graded strength take up water from the weaker and lose it to the stronger solutions. Suction pressure is equal to the osmotic pressure of the solution with which no exchange takes place. With increasing concentration above this point water loss increases till the cells have lost all turgor, and at still higher concentrations plasmolysis takes place. But, as the solution now enters the space between wall and protoplast, no further loss of water from the tissue occurs and there is no greater loss of weight. Percentage loss of weight plotted against concentration gives a straight sloping line which bends sharply to the horizontal at the concentration just causing plasmolysis. The point of inflection indicates the osmotic pressure of the tissue as measured by incipient plasmolysis. This may be verified by miscroscopical examination.

This describes the course of an experiment with potassium nitrate solution. On one occasion sucrose was used with quite different results. The graph did not bend to the horizontal at the point of plasmolysis but continued sloping at a decreased angle. Even with plasmolysed cells there was increasing water loss from the tissue as a whole and therefore shrinkage of the discs. This was evident in their appearance; they were shrunken, with concave faces, while in strong solutions of potassium nitrate they remained quite flat.

I am indebted to Miss M. E. Barton for carrying out a careful set of determinations the graphs for which are given in Fig. 1. The suction pressure of the tissue is just below 0·2 M sucrose to which water loss is only 1 per cent. For potassium nitrate the graph for a set of determinations made after 2 hours bends sharply at 0·4 M; at this concentration the cells are just plasmolysed. There is no greater loss of weight at higher concentrations and even an indication of a gain. This might be expected, as the space between wall and protoplast is occupied by a solution denser than water. The sucrose graph at 2 hours is a straight line with no bend at 0·6 M, the concentration at which plasmolysis takes place. At 26 hours there is a sharp bend at this point, though the graph continues to rise; at 72 hours this portion of the graph is nearly horizontal.

These observations are explained if the wall is readily permeable to potassium nitrate but offers a high resistance to the passage of sucrose. The sucrose solution withdraws water from the cell as a whole, the wall acting as a semipermeable membrane, though a very imperfect one. After prolonged immersion of the discs reabsorption of water, or solution, takes place, largely as the result of what may be called the *resilience* of the cell walls. The discs recover from their shrunken appearance and nearly resume the original volume. The greater resistance to sucrose is also indicated by the fact that equilibrium is attained in potassium nitrate in 2 hours, while in sucrose more than 26 hours is necessary. That the effect is due to the cell wall is shown by the fact that discs killed by chloroform or by boiling water and washed free of

solutes lose water to begin with, both to salts and to sucrose solutions, and later take it up again.

3. EXPERIMENTAL

The use of dead tissue simplifies the conditions, as only the wall can be effective. The small amount of coagulated plasma may have some adsorptive
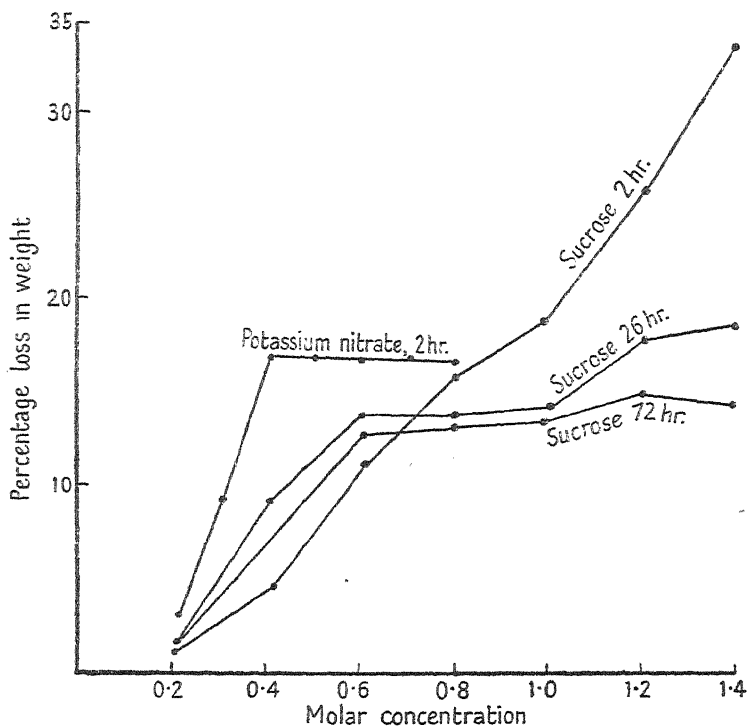
FIG. 1. Loss of weight of discs of beet in solutions of potassium nitrate and sucrose.

action, but no evidence has emerged to indicate that this is appreciable. If change in volume as well as change in weight is measured, it is possible to follow the course of the diffusion of the solute into the tissue and this gives a basis for a quantitative treatment. For suppose a set of discs in a solution has diminished by a volume equivalent to A gm. of water, and has lost B gm. in weight, then $A-B$ is a measure of the gain in solute. To this value a correction must be applied for the change in volume which takes place when a substance goes into solution. When a solute diffuses into a disc containing water, either the volume must increase or water must be expelled. In either event the measurements are affected. This change in volume varies both with the solute and with the concentration. For each solute used determinations were made of volume change on solution at a number of concentrations. The appropriate corrections were made by the method given in the Appendix. The

correction may be very large; for example, a measured gain in solute of 1 gm. may be equivalent to a movement of sucrose of more than 2 gm.

Initial weight and volume were determined by weighing the discs in a Hubbard pycnometer, filling up with water and reweighing. Weight of pycnometer and weight filled with water being known, weight and volume of discs are readily calculated. Subsequent weights and volumes were determined in the same way using the solution instead of water. The density of the solution was determined for each experiment; an example of the calculation is given in the Appendix. Although *volume* is referred to for convenience, real volumes need not be calculated. It is *weight* of water equivalent to the change in volume which is required, and this weight is estimated directly by the displacement method. All determinations were made at constant temperature and no temperature corrections are necessary.

Two main sets of experiments were carried out. In the first, discs of beet, killed in boiling water, were used and weighings were made to 5 mg. on a student's balance. In the second, in order to avoid any possible effects of high temperature on the condition of the cell wall, the discs were killed in chloroform. For this set an aperiodic balance was available and rapid weighings to 1 mg. could be made. The results of the two sets agree and only the second, and more accurate set, need be dealt with.

The course of an experiment was as follows. Discs of beet 2 mm. thick were cut from cylinders punched out with a cork-borer 1·9 cm. in diameter. This thickness was found best, as thinner discs give changes which are too small and thicker take too long to come to equilibrium. The discs were soaked in chloroform water for an hour, washed in running water overnight, and transferred to distilled water. They were free from anthocyan and sugars, and were fairly firm though easily deformed by pressure.

The pycnometer was then weighed empty and filled with distilled water at 20° C. Ten discs were removed from the water, placed on a sheet of blotting-paper, covered, but not pressed, by a second sheet; the whole was reversed, the first sheet removed and replaced by a third. The dried discs were placed in the dry pycnometer and weighed; the pycnometer was filled up with distilled water and weighed. The discs were again dried and dropped into 500 ml. of the solution in a large beaker, placed in a bath at 20° C. They were stirred by a stream of moist air bubbled through the solution. The moisture in the air stream partly balanced evaporation and the loss in weight over 8 hours was not more than 1 per cent. The volume of the solution was large compared with that of the discs, so that change in concentration due to exchange with the discs was negligible. After an appropriate interval the solution was poured through muslin supported over a beaker. From the muslin the discs could be rapidly removed by a spatula, dried, and weighed in the pycnometer in air and in the solution employed. The discs were then returned to the solution and determinations repeated at intervals.

There are several sources of error. Though the same lot of beet was used throughout there is variation from tuber to tuber; and within a tuber there is

variation in the discs from the varying amount of vascular tissue which represents open holes. As far as possible discs with a small amount of vascular tissue were chosen. Then there is an error in the method of drying. Living, turgid discs are very firm and can be pressed dry. But killed discs owe what firmness they have to the resilience of the walls alone and they are easily deformed by pressure; consequently this should be no more than the weight of the blotting-paper. This makes for less even and efficient drying. Because of this, and of slight deformations, a constant end weight is not usually attained. There is also some uncertainty as regards the timing. There is no definite point during the drying process at which we can say that absorption of the solute has ceased. It has been assumed that after the second blotting-paper is placed on the discs absorption is negligible until the pycnometer is filled up with solution. This interval was kept at $1\frac{1}{2}$ minutes and allowance has been made for this in the times given in the tables. It will be seen that a high degree of accuracy is not to be expected. Yet the results of duplicate sets show very fair agreement and fall into regular order.

The solutes used, all in molar solution, were sucrose, glucose, glycerol, magnesium sulphate, calcium chloride, potassium nitrate, potassium chloride, and sodium chloride. The data for one experiment with each solute are given in Tables I to VIII (see Appendix).

4. DISCUSSION

An examination of the data for volume shows that in all solutions the tissue loses water to begin with. The wall is therefore to some extent semipermeable to all solutes. If we compare the water losses for those solutions with equal osmotic pressure, sucrose, glucose, and glycerol, we find that the mean maximum losses are 39, 13, and 2 per cent. The semipermeability is differential and the series agrees with the series of diffusion coefficients which may be taken as an indication of molecular size. This suggests, as we might expect, that we are dealing with a membrane exercising a sieve effect.

The loss of water goes on for longer with the larger molecules; for sucrose it proceeds for about 45 minutes and with glycerol for about 13 minutes. Reabsorption is slow and is incomplete in solutions to which much water is lost. Thus in sucrose the discs attain only 87 per cent. of their original volume. This is probably because, with considerable contraction, the cell walls are so much deformed that their resilience is unable to bring them to their original shape.

More exact information is given by the values for absorption of solute. In the last columns of Tables I–VIII are given the amounts of absorption (millimoles) by ten discs; graphs showing the course of absorption for three solutes are given in Fig. 2. If we were dealing with diffusion into a constant volume of liquid it would be possible to give a mathematical formulation of these results, but here the conditions are very much more complex. The change of the diffusion gradient is caused not only by movement of the solute but also by

water movement; and this is outwards to begin with and later inwards; further the inward movement is probably in part a mass movement of solution. The rate of water movement is determined partly by the concentration gradient and partly by the resilience of the walls; this latter component opposes movement to begin with and favours it later. But it is possible to derive values for
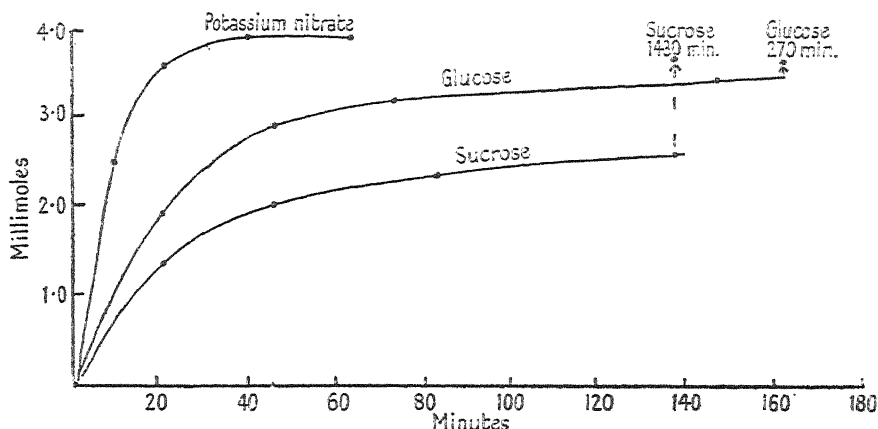
Fig. 2. Diffusion into discs of beet of sucrose, glucose, and potassium nitrate.

rate of absorption from the graphs of absorption plotted against time. In a large-scale graph the slope of the tangent to the curve at zero time can be determined. This gives initial rate of absorption in millimoles per minute. As a check on the values so determined we can also determine from the graphs the time for half the total absorption. No precise significance can be attached to these 'half-reaction times', but they serve as a check. In Table IX are given all the values of initial absorption rate for the set of experiments considered, with their means, and the half-reaction times with their means. In Table X are given the diffusion constants with their ratios, the mean initial rates with their ratios, and the mean half-reaction times with the ratios of their reciprocals. Diffusion constants have been taken from the International Critical Tables and have been corrected where necessary for temperature and concentration. It is not possible to get a set of strictly comparable values as different investigators have used different methods and conditions.

The values for initial rate are confirmed by those for half-reaction time. The ratios of the two fall in the same order and are numerically close together. It may be noted that one of the half-reaction times for sucrose is aberrant. If it is neglected the ratios are almost identical, that for potassium chloride, for example, coming out at 7·9. These two sets of values also fall in the same order as the diffusion coefficients, but their ratios rise much more steeply. Part of the observed effect is due to differences in rate of diffusion but there is clearly also a differential resistance.

Before attempting to make further use of the data it is necessary to consider

what precise meaning can be attached to the initial absorption rate. Theoretically the initial phase consists in the passage of solute through the outermost cell wall under a diffusion drop of 1 mole across that wall. It is quite unlikely that the method used can reveal this phase, for it must be of very short duration; even with sucrose, one-third of the final value is reached within 10 minutes. It is therefore more reasonable to assume that we are dealing with diffusion throughout the tissue from both sides; that the initial gradient is 1 mole over 1 mm. (half the thickness of the disc), and that resistance is offered by 10 walls, as the disc is on the average 20 cells thick. Even so it is likely that the rates are too low in the absence of data nearer the starting-point.

Working on this basis we can calculate rates of diffusion in moles per cm.^{2} per second for a gradient of 1 mole over 1 cm. and through 1 cell wall. These values are expressed in the same terms as diffusion coefficients. If we take them as representing diffusion through water with the added resistance of 1 cell wall, we can get at least a rough indication of the increased resistance offered when a cellulose wall is interposed in the path of diffusion. The resistance is increased about 80 times for sucrose, 60 for glucose, 50 for glycerol, and 40 for potassium nitrate and potassium chloride. As the diffusion coefficient of potassium chloride in 10 per cent. gelatin is about three-quarters of that in water, the resistance of the cellulose wall is about 30 times that of a stiff gelatin gel.

The initial rates also allow us to make some comparison between the resistance of the protoplasm and that of the wall. Several investigators have recently made estimates of the permeability of the protoplast in absolute terms by means of the deplasmolytic method. Hofmeister (1935) has done this for nine different plants and a number of organic compounds. Wahry (1936) compared air- and water-leaves of Hippuris in light and dark, using organic compounds and potassium nitrate. From the data given by these two investigators we can calculate values in the terms we are using. Permeability varies greatly from one species of plant to another and also in different conditions. To get values for a general comparison the means of the nine plants used by Hofmeister have been taken and these, with Wahry's values for Hippuris water-leaves in light, are given in Table XI, along with diffusion constants and the wall permeability values. Values are given only for those compounds common to the present investigation and to those of Wahry and Hofmeister.

Comparing the cell wall with the protoplast it appears that the resistance of the latter is from 50,000 to 80,000 times as great for sucrose, 50,000 to 60,000 for glucose, 1,500 to 7,000 for glycerol, and 14,000 for potassium nitrate. So that, while the cell wall offers a definite and differential resistance to diffusion, it is clear that in ordinary conditions of cellular exchange it will have little effect, since the protoplasmic permeability is so much lower. But in physiological experiments of certain types wall-resistance may be effective. This is seen if we consider the permeability of the protoplast to water; Wahry's figure is given in Table XI. It is only about one-sixth that of the wall per-

meability to sucrose. Thus in experiments on rates of plasmolysis or deplasmolysis one would expect to find the wall-resistance exercising an effect when sucrose is used as the plasmolyticum. This is precisely what Höffler found when using cells with low permeability to water, plasmolysed in sucrose solutions.

SUMMARY

1. It is shown that the thin, unaltered cellulose wall offers resistance to the passage of solutes.

2. A method is described for the estimation of this resistance.

3. Comparing the cell wall with the protoplast, it appears that the resistance of the latter is from 50,000 to 80,000 times as great for sucrose, 50,000 to 60,000 for glucose, 1,500 to 7,000 for glycerol, and 14,000 for potassium nitrate.

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APPENDIX

Data of Table II, line 3, are used here to show the methods of calculation.

1. *Calculation of Volume of Disc.*

Wt. of pycnometer	29·286 gm.
Wt. of pyc. + water	54·593 „
Wt. of water	24·727——A
Wt. of pyc. + glucose sol.	56·285 gm.
Wt. of glucose sol.	26·419——B
Density of glucose sol., B/A	1·068——C
Wt. of pyc. + beet	34·199——D
Wt. of beet	4·333 gm.
Wt. of pyc. + beet + glucose sol.	56·300——E
Wt. of glucose sol., $E–D$	22·101——F
Wt. of glucose sol. displaced $B–F$	4·318——G
'Volume' of beet, i.e. weight of water equivalent to glucose sol. displaced, G/C	·0 ml. (gm.)

2. *Calculation of Weight of Solute which has entered the Discs.*

Initial weight of beet	4·675 gm.
Weight of beet at 23½ minutes	4·333 ,,
Loss of weight	0·342——*A*
Initial volume of beet	4·614 ml.
Volume of beet at 23½ minutes	4·042 ,,
Loss of volume	0·572——*B*
Weight loss deficit, which may be called the *measured weight* of glucose which has entered the discs, *B-A*	0·230 gm.

Apparent concentration of glucose in discs $\dfrac{0·23 \times 100}{4·042} = 5·7$ per cent.

But the entry of glucose means an increase in volume, or a loss of water, and this must be allowed for. The increase in volume when glucose goes into solution may be determined by adding a known weight of glucose to a known volume of water in a burette and noting the change in volume. The following values were obtained for glucose which was added to 50 ml. water in each case:

Wt. of glucose.	Vol. increase.	Vol. increase per gm. glucose.	Concentration.
3 gm.	1·5 ml.	0·5 ml.	5·8 per cent.
6 ,,	3·4 ,,	0·566 ,,	11·2 ,,
9 ,,	5·2 ,,	0·577 ,,	16·3 ,,

We cannot make use of these values directly, for we do not know the *real concentration* of glucose in the discs, but only the *apparent concentration*, derived from the *measured weight* of glucose entry. The values are converted into useful form as follows.

If 3 gm. glucose is added to 50 ml. water the volume increases to 51·5 ml. Consider this volume to consist of two parts, of 50 ml. and 1·5 ml. Then the glucose is distributed 2·91 gm. in the 50 ml. part and 0·09 gm. in the 1·5 ml. part. The water is distributed 48·57 ml. in the 50 ml. part and 1·43 ml. in the 1·5 ml. part. In the 50 ml. part 2·91 gm. glucose has gone into solution and has expelled 1·43 ml. water. In this part there has therefore been a gain of 2·91 gm. and a loss of 1·43 gm., that is there has been a net gain of 1·48 gm. This corresponds to the *measured weight* of glucose entry and from it we can derive the *apparent concentration* of glucose in the burette as

$$\frac{1·48 \times 100}{50} = 2·96 \text{ per cent.}$$

We can now say that at an *apparent concentration* of 2·96 per cent. a *measured weight* of glucose of 1·48 gm. is equivalent to a *real weight* of 2·91 gm. By treating the other values in the same way we can put our information in the useful form:

1 gm. *measured weight* of glucose is equivalent to:

2·004 gm. *real weight* at an *apparent concentration* of 2·96 per cent.				
2·314 ,,	,,	,,	4·86 ,,
2·368 ,,	,,	,,	6·89 ,,

The values for real weight are plotted against apparent concentration. For each determination the apparent concentration is calculated, and from the graph there can be obtained, for this concentration, the real weight of solute entering the discs for 1 gm. measured weight. Thus, for the example we have been using, the apparent concentration is 5·7 per cent. At this 1 gm. measured weight is equivalent to 2·35 gm. real weight. The measured weight is 0·23 gm. and is equivalent to 0·5405 gm. or 3·002 millimoles.

TABLE I

Absorption of Sucrose

Time (min.).	Wt. of discs (gm.).	Vol. of discs (ml. (gm.)).	Net wt. gain (gm.).	Amount absorbed (mmol.).
0	4·701	4·658	—	—
10	3·695	3·450	0·202	1·434
23	3·258	2·928	0·287	2·059
41·5	3·254	2·871	0·340	2·444
70	3·379	2·962	0·347	2·688
129·5	3·617	3·171	0·403	2·897
254	3·925	3·436	0·446	3·205
347·5	4·101	3·591	0·467	3·357
1405	4·723	4·152	0·528	3·795
1430	4·719	4·147	0·529	3·803

TABLE II

Absorption of Glucose

Time (min.).	Wt. of discs (gm.).	Vol. of discs (ml. (gm.)).	Net wt. gain (gm.).	Amount absorbed (mmol.).
0	4·675	4·614	—	—
10	4·368	4·144	0·163	1·966
23·5	4·333	4·042	0·230	3·002
37	4·407	4·092	0·254	3·329
75·5	4·595	4·263	0·271	3·559
136	4·757	4·408	0·288	3·790
173·5	4·803	4·453	0·289	3·803
199	4·833	4·478	0·294	3·868
273·5	4·869	4·516	0·292	3·843

TABLE III

Absorption of Glycerol

Time (min.).	Wt. of discs (gm.).	Vol. of discs (ml. (gm.)).	Net wt. gain (gm.).	Amount absorbed (mmol.).
0	4·698	4·638	—	—
5	4·683	4·569	0·054	2·030
13·5	4·705	4·559	0·086	3·391
27	4·737	4·583	0·094	3·717
40·5	4·753	4·596	0·097	3·856
103	4·754	4·591	0·103	4·118
164·5	4·748	4·583	0·105	4·220

TABLE IV

Absorption of Magnesium Sulphate

Time (min.).	Wt. of discs (gm.).	Vol. of discs (ml. (gm.)).	Net wt. gain. (gm.).	Amount absorbed (mmol.).
0	4·579	4·528	—	—
10	3·971	3·699	0·221	1·957
18·5	3·865	3·528	0·286	2·577
32	3·900	3·508	0·341	3·113
80·5	4·120	3·680	0·389	3·576
200	4·373	3·904	0·418	3·847
257·5	4·486	4·003	0·432	3·979
300	4·538	4·054	0·433	3·985
325·5	4·573	4·082	0·440	4·053
1464	4·720	4·217	0·452	4·159
1502	4·772	4·285	0·436	3·996

TABLE V

Absorption of Calcium Chloride

Time (min.).	Wt. of discs (gm.).	Vol. of discs (ml. (gm.)).	Net wt. gain (gm.).	Amount absorbed (mmol.).
0	4·698	4·645	—	—
5·5	4·668	4·390	0·225	2·463
15	4·759	4·381	0·325	3·558
25	4·827	4·414	0·360	3·940
35	4·876	4·454	0·369	4·038
50	4·906	4·475	0·378	4·137
65	4·950	4·509	0·388	4·246
80	4·942	4·508	0·381	4·170

TABLE VI

Absorption of Potassium Nitrate

Time (min.).	Wt. of discs (gm.).	Vol. of discs (ml. (gm.)).	Net wt. gain (gm.).	Amount absorbed (mmol.).
0	4·591	4·549	—	—
4	4·753	4·539	0·172	2·619
10·5	4·821	4·535	0·244	3·746
20	4·846	4·544	0·260	4·012
32·5	4·843	4·537	0·264	4·073
46	4·848	4·541	0·265	4·088

TABLE VII

Absorption of Potassium Chloride

Time (min.).	Wt. of discs (gm.).	Vol. of discs (ml. (gm.)).	Net wt. gain (gm.).	Amount absorbed (mmol.).
0	4·837	4·774	—	—
3	4·896	4·707	0·126	2·544
8·5	4·932	4·695	0·174	3·524
17	4·961	4·699	0·199	4·057
30·5	4·970	4·700	0·207	4·235
44	4·994	4·724	0·207	4·235
57·5	4·983	4·713	0·207	4·235

TABLE VIII

Absorption of Sodium Chloride

Time (min.).	Wt. of discs (gm.).	Vol. of discs (ml. (gm.)).	Net wt. gain (gm.).	Amount absorbed (mmol.).
0	4·479	4·443	—	—
5	4·525	4·354	0·115	2·676
15·5	4·575	4·363	0·156	3·629
23	4·598	4·373	0·169	3·932
36·5	4·621	4·394	0·171	3·978
100	4·599	4·373	0·170	3·954

TABLE IX

Initial Absorption Rates and Half-Reaction Times

Solute.	Initial rate (mmol./min.).	Mean.	Half-reaction time (min.).	Mean.
Sucrose	0·19, 0·20, 0·21	0·20	18, 19, 25	20·6
Magnesium sulphate	0·27, 0·30, 0·31	0·29	11·0, 11·5, 12·5	11·7
Glucose	0·35, 0·36, 0·37	0·36	9·0, 9·5, 9·5	9·3
Glycerol	0·56, 0·58, 0·66	0·60	4·5, 5·0, 6·5	5·2
Calcium chloride	0·84, 0·90 —	0·87	4·0, 4·5	4·25
Sodium chloride	1·0, 1·15, 1·25	1·14	2·8, 3·3, 3·3	3·2
Potassium nitrate	1·25, 1·35, 1·45	1·35	2·6, 2·75, 2·8	2·71
Potassium chloride	1·5, 1·75 —	1·60	2·1, 2·6	2·35

TABLE X

Rates and Ratios of Diffusion and Absorption

Solute.	Diff. coeff. ($\times 10^{-5}$).	Ratios.	Initial Rate.	Ratios.	Half-reaction time (min.).	Reciprocal of Ratios.
Sucrose	0·38	1·0	0·2	1·0	20·6	1·0
Magnesium sulphate	0·46	1·21	0·29	1·45	11·7	1·76
Glucose	0·6	1·58	0·36	1·8	9·3	2·22
Glycerol	0·77	2·02	0·6	3·0	5·2	3·96
Calcium chloride	0·98	2·58	0·87	4·35	4·25	4·85
Sodium chloride	1·25	3·29	1·14	5·7	3·17	6·5
Potassium nitrate	1·35	3·55	1·35	6·75	2·75	7·5
Potassium chloride	1·61	4·25	1·6	8·0	2·35	8·8

TABLE XI

Diffusion Coefficients, Initial Rates, and Protoplasmic Permeability (*Mol. per sec. per cm.*^{2})

Solute.	Diff. coeff.	Initial rate.	Protoplasmic Permeability: Hofmeister.	Wahry.
Sucrose	$3·8 \times 10^{-6}$	$4·9 \times 10^{-8}$	$9·1 \times 10^{-13}$	$5·9 \times 10^{-13}$
Glucose	$6·0 \times 10^{-6}$	$8·8 \times 10^{-8}$	$1·7 \times 10^{-12}$	$1·4 \times 10^{-12}$
Glycerol	$7·7 \times 10^{-6}$	$1·5 \times 10^{-7}$	$1·1 \times 10^{-10}$	$2·0 \times 10^{-11}$
Potassium nitrate	$1·35 \times 10^{-5}$	$3·3 \times 10^{-7}$	—	$2·1 \times 10^{-11}$
Potassium chloride	$1·61 \times 10^{-5}$	$3·9 \times 10^{-7}$	—	—
Water	—	—	—	$8·0 \times 10^{-9}$

Studies on Germination and Seedling Growth

II. The effect of the Environment during Germination on the subsequent Growth of the Seedling of Barley

BY

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With seven Figures in the Text

INTRODUCTION

WHEN the embryo of the cereal grain is separated from the endosperm, it will grow and with suitable treatment it may develop into a mature plant. The development of the isolated seedling is, however, profoundly affected by the time after germination of the intact seed at which the excision is made. Schander (1934) found that maize seedlings excised at 24 hours after germination had commenced sustained a more vigorous growth than others that had been removed from dry seeds. De Ropp (1939) compared the linear growth of rye seedlings taken from dry seeds and from seeds that had been soaked for 2, 8, 24, and 48 hours. He reported that both coleoptile and root lengths increased with soaking time from o to 8 hours, and decreased from 8 to 48 hours. Clearly at least during the earlier stages of germination of the intact seed conditions are established which stimulate the subsequent growth of the seedling. Both Schander and De Ropp attributed the stimulation to the absorption by the seedling of accessory growth factors. Other factors,

however, may also be involved, since separation of the seedling from a source of growth hormones is not the only consequence of early excision. The present investigation (Brown, 1943) has shown that the environmental conditions within the seed differ in several important respects from those of the artificial culture medium to which the embryo is transferred. Thus during the early critical period of germination in the intact seed the seedling is exposed to a low level of water availability, and to a substrate in equilibrium with a partial pressure of carbon dioxide, which during certain periods exceeds 0·1 atm., and with a partial pressure of oxygen which is about 0·1 atm. When the embryo is transferred to an artificial culture medium the conditions change; usually the level of water availability increases and the concentrations of oxygen and carbon dioxide rise and fall respectively. Moreover, in the seed the embryo is in contact with water and not with a nutrient solution; at this stage most of the nutrients of the endosperm are insoluble. Contact with a nutrient solution containing both inorganic salts and sugars constitutes in itself an important environmental change.

The present investigation involves two sets of experiments. Schander's results were reported in terms of general appearance, De Ropp's striking observations involved linear measurement. One group of experiments in the present series is therefore designed primarily to determine the effect of the time of excision after water uptake begins on another aspect of growth, the subsequent rates of fresh and dry weight increase. The elucidation of the origins of these effects is the purpose of the second group of experiments. In the seed the seedling is exposed to one set of environmental conditions; in the culture medium to another. The different effects of early and late excision may therefore be due to the conditions of the seed or to those of the culture medium operating during the same time and therefore during the same phase of development. The problem is investigated here by comparing the effects of exposure of the seedling to different intensities of each of several factors whose levels change when the embryo is transferred from the seed to the artificial culture medium; the exposure is made during a period which corresponds to the different times of excision within which the primary effects are established. The growth reactions, as in the first group of experiments, are measured in terms of fresh and dry weight. In both experimental series the weight measurements are supplemented by linear measurements of coleoptiles and first leaves.

De Ropp found that seedlings excised at 8 hours grew better subsequently than others excised at 2 hours. Schander adduced evidence that during the first 6 hours of germination effects were established which stimulated the subsequent growth of the seedling. The results of the first group of experiments in the present series showed that the subsequent growth rate increased as the time of excision after contact with water by the whole seed increased from 2 to 6 hours. Thus, for the second group of experiments designed to establish the origin of this effect, seedlings were excised at 2 hours after germination began. They were exposed to a known experimental environ-

ment for 6 hours, after which they were transferred to a standard culture medium in which the subsequent growth reaction was observed. The effects of several levels of a number of factors in the artificial environment on the subsequent growth rate could therefore be compared with the corresponding effects of excision time.

<center>EXPERIMENTAL METHODS</center>

The seedlings, whatever the experiment, must be placed ultimately in a standard culture medium in which the growth reaction is observed. Throughout this investigation the growth measurements are made primarily in terms of fresh and dry weight increase, and the form of the experimental technique is determined largely by this condition. These measurements necessitate the selection of serial samples at suitable intervals. Unfortunately, the variability of the material is large, and the population on which the observations are made must be correspondingly large. But two other conditions must also be fulfilled: carbohydrates must be supplied in the culture medium, and the seedlings must be kept comparatively free from bacterial contamination. Several investigators (Brown and Morris, 1890; Barnell, 1937; James and James, 1940) have shown that starvation symptoms develop rapidly when cereal embryos are cultured without carbohydrates. The incorporation of these, however, renders the medium liable to massive bacterial contamination. This condition it is necessary to avoid since the irregular incidence of bacteria involves using media of variable composition and of uncertain hydrogen-ion concentration, and the roots of seedlings growing in heavily contaminated media are frequently covered with a thick bacterial slime. Complete sterility would therefore be the ideal cultural condition, but the exigencies of the experimental situation render it impossible. Complete cultural sterility would require an intensive sterilization of the soaked grain, and it is very doubtful if this can be achieved without some injury to the embryo. Moreover, in some of these experiments two series of transfers instead of one are involved; this promotes a high degree of infection. The large size of the experimental population also contributes to the difficulties of sustaining completely sterile conditions. The experimental procedure is therefore not designed to ensure complete sterility, but a variety of measures is incorporated into the technique to reduce bacterial contamination and to avoid altogether massive infection. The incidental contamination which the method used necessarily involves has little or no effect on the growth of the seedlings. All the apparatus is sterilized in an autoclave, and all the media are also completely sterilized, but the parent seeds are treated only with absolute alcohol for 2 minutes before they are brought into contact with sterile water to induce germination. The manipulative instruments are flamed, but the transfers are not made in a sterile atmosphere. Various devices are incorporated into the cultural apparatus to reduce the intensity of infection.

The whole investigation involves four sets of techniques, each appropriate

to one of the following operations: (1) inducing water uptake by the whole seed; (2) excising the embryo; (3) providing the intermediate artificial environment; (4) culturing the isolated embryos.

Before excision of the embryos the parent grains absorb water for a variable period which is never less than 2 hours. During this time they are not completely immersed but are floated on a free water surface. In these circumstances the grains are in immediate contact with free water, and the gaseous exchange is not as restricted as it would be if the grains were submerged. The construction of the floats is described elsewhere (Brown, 1943).

It is particularly important that the seedlings should be excised without injury. No satisfactory method has been devised by the present investigator for fulfilling this condition with excisions from the dry seed, but when the seed has taken up water for 2 hours undamaged embryos can be excised from it consistently. Hence the preliminary exposure to water for at least 2 hours. No surface injury can be seen, and experimental evidence presented in a later section confirms the suggestion that embryos excised at this stage are in fact undamaged. The technique of excision is described elsewhere (Brown, 1943).

As indicated above, when the embryo is transferred from the seed to a culture medium changes in the levels of at least four factors are involved: oxygen, carbon dioxide, water, and nutrient. The effects of the different levels of each of these factors are examined by exposure of the seedlings excised at 2 hours to different artificial environments for 6 hours. The artificial environments are established in cylindrical glass vessels of about 300 c.c. capacity provided with rubber bungs traversed by two glass tubes through which the composition of the atmosphere in the vessel is adjusted. Small dishes rest on the floor of the vessels and these hold the several media on which the seedlings are placed. The effects of four levels of water availability are compared by placing the embryos on water, 10, 20, and 30 per cent. gelatine. The embryos are floated on the water in the manner already described for intact seeds. Gelatine is more suitable than agar, since there is apparently some free water on the surface of the agar gel whatever the concentration; on this medium water uptake is therefore only slightly less than it is in pure water. The comparative effects on growth of water and agar have also been examined. The results which are presented in a later section, Table II, show that the two media do not have markedly different effects.

The effects of different partial pressures of oxygen and of carbon dioxide are investigated by exposing different series to three concentrations of carbon dioxide, 20, 10, and 0·3 per cent. (air), and four of oxygen, 20 (air), 15, 10, and 5 per cent. In the oxygen experiments an anaerobic series is also included in which the embryos are exposed to free nitrogen. The gas mixtures were prepared by a volumetric displacement of water in a 12-litre bottle, cylinder gases being used throughout.

A comparison of the growth of seedlings transferred immediately to a culture solution and of others given a preliminary treatment of 6 hours in

water provides an indication of the effect of available nutrients during the early phases of development.

The culture medium to which the seedlings are transferred consists of a mineral nutrient (adapted from a mixture that was found by Gregory and Richards (1929) to be suitable for barley) dissolved in one litre of 2 per cent. sucrose. The solution is adjusted colorimetrically to pH 6·8. In these experiments the seedlings are bathed by the liquid medium, primarily for the reason that it is difficult to remove seedlings from a solid medium without injury to the roots and without adherent fragments, since it is clearly necessary that these two conditions should be fulfilled if adequate dry weight data are to be obtained. Moreover, preliminary experiments conducted under sterile conditions have shown that seedling growth is consistently better in liquid media in which the seedlings are not immersed than it is in sand, gelatine, or agar.

In the technique used in these experiments a sterile liquid medium is drawn through a culture vessel in a continuous current of small volume, the vessel being at the same time rocked slowly backwards and forwards. The roots throughout the experiment are bathed by an uncontaminated liquid medium whose composition does not change with time. The rocking ensures a free gaseous diffusion and prevents the establishment of stable films of liquid between and around the roots in which bacteria might develop. Freedom from massive infection cannot, however, be maintained with this technique for more than 5 days, and the method is not suitable for experiments lasting longer. For longer periods the method of James and James (1940) is clearly preferable.

The apparatus used consists of a series of separate units, one of which is shown in Fig. 1 resting on a platform which is rocked by the device shown in Fig. 2.

In Fig. 1 the platform is shown in section at A. It supports the culture vessel B, which is secured to it by a rubber band C. The rubber band is stretched over the culture vessel and attached to two hooks screwed into the platform on either side of the vessel. The culture vessel consists of a glass tube having a diameter of 1·5 in. and a length of 9 in., and bent into the shape of a flat V. The culture medium is introduced through the tube D and withdrawn through the tube E. E is bent at right angles within B and drawn to a fine point which touches the lower surface of the culture vessel. The culture vessel is in direct communication with the air through the tube F, the free portion of which is packed with cotton-wool. The tubes F, D, and E traverse rubber bungs. G is a 250-c.c. conical flask acting as a reservoir of sterile culture fluid. The neck of the flask is provided with a rubber bung which is traversed by two tubes H and I. H above the flask is packed with cotton-wool, and its upper end is secured to a short length of pressure tubing J. A short length of copper wire runs along the channel of J, the cross-sectional area of which can be controlled by the screw clip L. The whole system above H constitutes a simple gas flow regulator. I is connected to D on the culture vessel through a length of

thin-walled rubber tubing T. The conical flask G is raised above the level of B and thus fluid siphons into the culture vessel through I, T, and D, the rate of flow being controlled by the screw clip L.

N is a 250-c.c. conical flask into which the culture fluid is withdrawn from the culture vessel. The neck of N is provided with a rubber stopper traversed
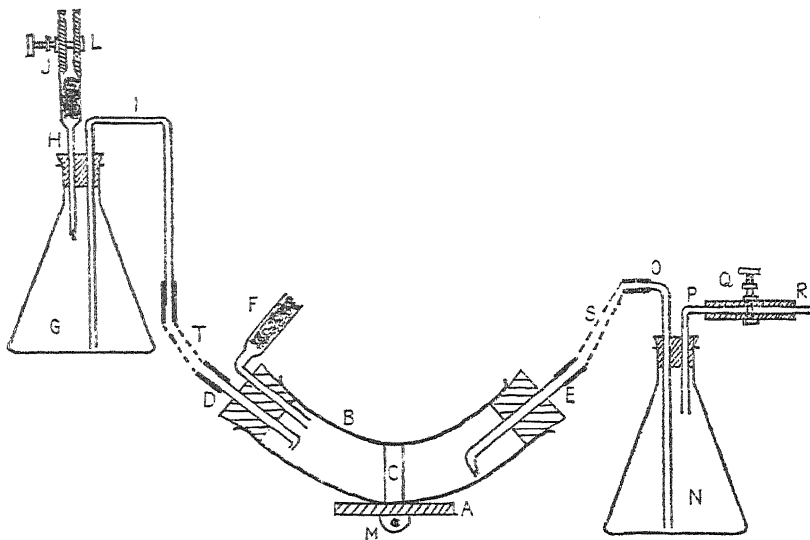
FIG. 1. Diagram of single unit of the apparatus for culturing embryos.
For explanation see text.

by two tubes O and P. P is connected to an air-flow regulator Q similar to that above H. The distal end of the length of pressure tubing at Q is attached to a tube R along which a reduced pressure is transmitted to N across Q. The reduced pressure is developed in a series of 12-litre vessels (not shown) out of which water siphons at a rate depending on the resistances established in the series of regulators corresponding to Q. O is connected to E through a length of thin-walled rubber tubing S. The flow of fluid and gas from the culture vessel into N is controlled by the regulator Q.

In Fig. 2 the platform is again shown in cross-section at A. A metal rod, shown in section at M (Figs. 1 and 2), is secured to the lower surface of A along its whole length. The two ends of M project beyond both ends of A and fit into metal sockets in which they can rotate freely. The lever B is attached transversely across one end of A. Lengths of cord C and D are attached each to an extremity of B. These pass over the rotating pulleys E and F. The second component of C is secured to an inverted conical flask G, the neck of which is closed by a rubber stopper traversed by a glass rod, which is in turn secured to a metal disc H which fits closely across a tall beaker I. G and H constitute a piston which traverses the length of I. At the other end of the system the cord D is secured to a beaker J. A composite tube K is suspended across the lip of J. K consists of two wide portions each welded to one end of a narrow tube bent

through 180°. The inner limb of K almost reaches the bottom of J, but the outer limb projects beyond the base of the beaker. L is a simple constant level device for ensuring a uniform flow of water into J.

When in operation water flows into J from L. As J fills the weight suspended from D increases until J falls and the end of B, to which D is attached, rises.
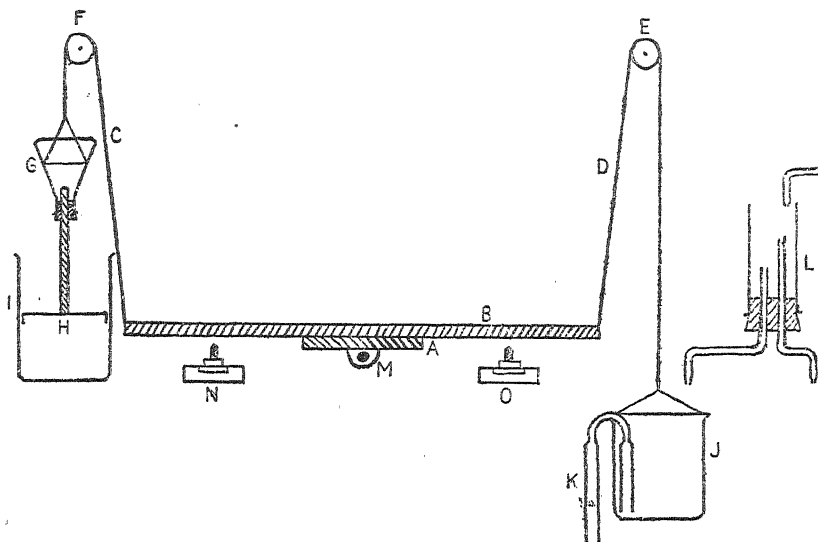
FIG. 2. Diagram of apparatus for rocking the culture vessel shown in Fig. 1.
For explanation see text.

J continues to fill and with it the inner limb of K. When the level in K reaches the narrow segment it rises above that in J. Thus immediately before the level in J reaches the lip water flows across the narrow portion of K and into the outer horizontal limb. A siphoning process immediately begins which removes water from J more rapidly than it is introduced from L. The weight of J decreases, and as it does so the D end of B in the system falls. Simultaneously the C end of B rises, and it does so under the influence of an appropriate weight established in G. The weighting of G is effected by placing in it lead shot.

It is necessary that the motions of the whole system should be slow to avoid mechanical disturbance to the seedlings. This condition is secured by adjusting the rates of inflow and outflow in J and by the damping effect of the water in I on the movements of the piston constituted by G and H.

The platform A accommodates eight units such as that of Fig. 1. The results of experiments with floats to hold the seedlings on the surface of a large body of liquid in the culture vessel have shown that this general method is not as satisfactory as the simpler arrangement of allowing the seedlings to rest on the floor of the vessel while maintaining in it only a very limited volume of liquid. The volume of liquid in the culture vessel can be regulated

by varying the amplitude of the swing by means of the variable stops below the lever B at N and O (Fig. 2), and by varying the position of the extremity of E in the culture vessel. In the present series of experiments the volume of liquid in the vessel at any one time never exceeds 2 c.c.

The culture medium in G is sterilized by filtering through a Seitz filter, not by heat. Filtering is more convenient for the purpose than heat sterilizing. The pH does not change and the elaborate precautions necessary when heat sterilizing a medium containing both sugars and mineral salts including phosphates are avoided.

Each experiment of the present series lasted 4 days. At intervals of 24 hours after the experiment is started samples consisting of 15 seedlings are taken. Two vessels, each of which holds about 65 seedlings, are allocated to each treatment. The results, given in a later section in terms of single embryos, are the means of two samples which together involve 30 seedlings. Data which indicate the accuracy that is attained by the cultural and sampling techniques are given in another section (Table I) together with the relevant conclusions.

The growth measurements made are those of fresh and dry weights and of lengths of coleoptiles and of first leaves. The seedlings immediately after being taken from the culture vessel are pressed between two layers of filter-paper; they are weighed, and then weighed again 24 hours later after heating in an oven kept at 80° C. The linear measurements are made immediately after the fresh weight has been determined. Linear measurements were only made on the final samples of each series. In the next section the fresh weight data are not given as such. They are replaced by the calculated values of the percentage water content based on dry weight, for the reason that these, as shown in the subsequent discussion, describe certain aspects of development more adequately than do the fresh weight data.

Each experiment throughout its whole course is conducted in a chamber in which the temperature is maintained at 22° C. $\pm$ 1° C.; the apparatus in which the seedlings are cultured being also operated in this chamber.

Each experiment is deemed to start when the parent seeds are brought into contact with water. Thus with experiments in which the variable is the time of excision after water uptake begins the first 24 hours of embryo growth includes the period of attachment to the parent seed. Similarly, with experiments in which the seedling is excised 2 hours after water uptake begins, and is then exposed for 6 hours to an artificial environment before being transferred to the culture medium, the first 24 hours of embryo growth includes both these preliminary periods. Certain terms used in this paper require some explanation. Excision at a certain number of hours means excision at that time after water uptake begins. With experiments in which there is an exposure to an artificial environment, the term pretreatment is applied, and it implies excision at 2 hours followed by treatment for a period of 6 hours before the seedling is transferred to the standard culture medium. The 'effects' observed refer to the influence of pre-treatment on subsequent growth.

The material used throughout these experiments (with one exception,

which is indicated in the text) is a sample of apparently well-matured Spratt Archer Barley obtained from a local firm of seed merchants.

EXPERIMENTAL RESULTS

Table I shows the results of three replicated experiments, each drawn from different series, which together indicate the adequacy of the experimental methods. The embryos were excised at 2 hours, and then pretreated by floating on water in contact with air.

TABLE I

A, B, and C represent Three Replicate Series. Embryos pretreated by Exposure to Air and Water. Data are Dry Weight (mg. per Embryo)

Age of sample. (hrs.)	A	B	C
24	1·32	1·41	1·54
48	1·76	1·90	1·88
72	2·35	2·41	2·65
96	3·08	3·02	2·90

The final values all agree within about 5 per cent. At earlier stages of growth, however, the aggregate weights being smaller the sampling errors are larger and the differences between replicate samples may exceed 10 per cent.

The effects of pretreatment with agar and water in air. A preliminary experiment to test the effect of agar as a supporting medium relative to that of water was undertaken. A 6 per cent. agar gel was used. The results are given in Table II in terms of dry weight.

TABLE II

A, Seedlings pretreated with Water; B, with 6 per cent. Agar. Both Series in Air. Data are Dry Weight (mg. per Embryo)

Age of seedlings. (hrs.)	A	B
24	1·44	1·42
48	1·97	2·12
72	2·60	2·55
96	3·00	3·23

There is a slight difference in favour of the agar series but it is not great. This result is no doubt due to the fact that water uptake on agar is almost as rapid as it is in water.

The effect of time of excision. Embryos were excised at 2, 4, 6, 8, and 10 hours and immediately transferred to the fluid nutrient medium without pretreatment. The dry weight data are given in Fig. 3a, the water contents in Table IV, and the lengths of the coleoptiles and first leaves of the final samples in Table III.

It is evident from Table III that growth in terms of dry weight tends to increase as the time of excision is increased from 2 to 6 hours, and that it then
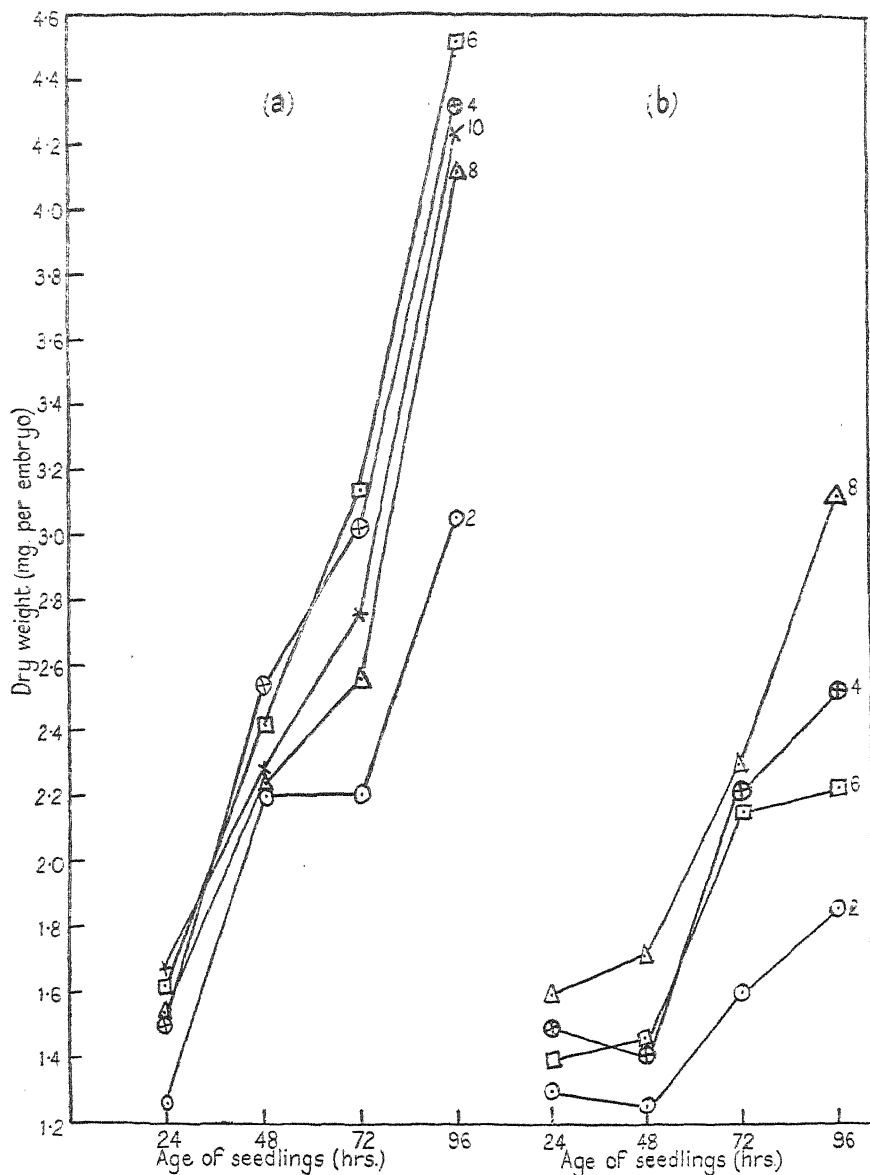
FIG. 3. Effect of time of excision on sub quent dry weight growth; (a) and (b) different asmples. Figures attached to curves indicate time of excision in hours after water uptake by whole seed begins.

tends to decrease as the time is still further increased from 6 to 10 hours. The difference between excisions at 2 hours and 4 hours is particularly striking.

TABLE III

Effect of Time of Excision on the Average Coleoptile and Leaf Lengths (cm.) of Seedlings in Final Samples taken at 96 Hours

Time of excision. (hrs.)	Coleoptile.	First leaf.
2	2·10	1·05
4	2·75	2·31
6	3·12	2·44
8	2·70	2·30
10	2·62	2·30

The effect of the time of excision on the final leaf and coleoptile lengths is relatively the same as it is on dry weight.

TABLE IV

Effect of Time of Excision on Water Content. Water Contents as Percentage of Dry Weight

Time of excision (hrs.)	2	4	6	8	10
Age of seedlings. (hrs.)					
24	515	515	366	328	289
48	692	730	645	646	744
72	1,190	984	770	1,070	1,012
96	1,122	1,040	1,016	1,065	1,024

At the end of the first 24 hours the water content decreases as the time of excision is postponed. Subsequently, however, the experimental variable has little if any effect on the water content.

It was clearly necessary to determine the extent to which the effects described above could be regarded as general for the seeds of this species. Accordingly the experiment was repeated with another sample of barley of the same variety, but obtained from another source. In this experiment dry weights only were measured. The results are presented in Fig. 3*b*. The general effect is the same. The later the time of excision, the greater tends to be the subsequent growth rate. Two salient differences between the results of the two series may, however, be noticed: the general forms of the growth curves are not the same, and corresponding dry weights tend to be much lower in this second series than they are in the first.

Since the experimental results showed that growth was less vigorous in this second sample than it was in the first, all subsequent experiments were made with the grain with which the results of Fig. 3*a* and Tables III and IV were obtained.

The effects of the level of water availability and the concentration of carbon dioxide in the atmosphere during pre-treatment. Some preliminary experiments

FIG. 4. Effect of level of water availability in substrate and of concentration of carbon dioxide in the atmosphere during pretreatment on subsequent dry weight growth: (*a*) air, (*b*) 10 per cent. carbon dioxide; (*c*) 20 per cent. carbon dioxide; w, water substrate, G indicates gelatine substrate, percentage concentration of which precedes G.

suggested an interaction between water availability and carbon dioxide concentration in pretreatment. Accordingly the effect of four levels of water availability were determined in three carbon dioxide concentrations.

The dry weight measurements of this series of experiments are presented in Fig. 4, the corresponding water contents in Table V, and the linear measurements of coleoptile and leaf lengths made on the final samples in Fig. 5.
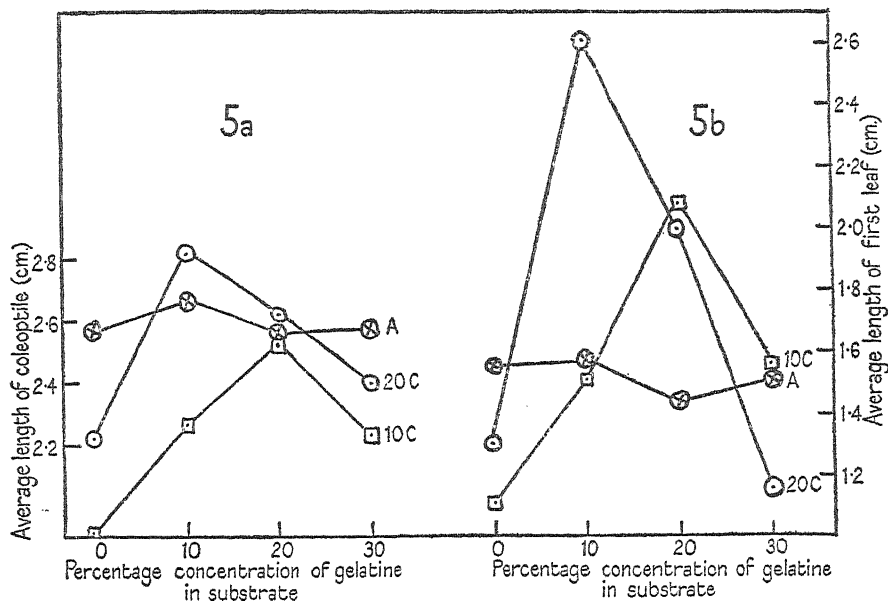
Fig. 5. Effect of level of water availability in the substrate and of concentration of carbon dioxide in atmosphere during pretreatment on average lengths of coleoptiles (*a*), and of first leaves (*b*), of seedlings of samples taken at 96 hours. Percentage concentration of gelatine in substrate along abscissae: o indicates water, A indicates air, 10 c indicates 10 per cent. carbon dioxide, and 20 c indicates 20 per cent. carbon dioxide.

The drift of dry weight with time is evidently very markedly affected both by the concentration of carbon dioxide and by the level of water availability to which they are exposed. Whatever the carbon dioxide concentration during pretreatment a higher growth rate is always obtained after exposure in 10 and 20 per cent. gelatine than after exposure to water and 30 per cent. gelatine. The effect of carbon dioxide is more complex. The series treated with 10 and 20 per cent. on 20 and 30 per cent. gelatine show a marked fall in the growth rate between 72 and 96 hours, and this tends to change the order of differences established at earlier stages; nevertheless, the general character of the carbon dioxide effect is sufficiently well shown by the final values recorded at 96 hours. In Fig. 6 the final dry weights reached with each carbon dioxide concentration are plotted against level of water availability. (The converse arrangement of plotting each water availability series against carbon dioxide concentration would display the carbon dioxide effect more vividly; but the form of Fig. 6

facilitates a comparison with Fig. 5.) With water and with 10 per cent. gelatine the seedlings exposed to 10 per cent. carbon dioxide achieve a lower, and those exposed to 20 per cent. carbon dioxide a higher, dry weight, than do, those exposed to air; with 20 per cent. gelatine carbon dioxide has apparently little effect; with 30 per cent. gelatine the dry weight decreases with increasing concentration of carbon dioxide.

In Fig. 5 the average coleoptile and leaf lengths given by the final samples at 96 hours at each carbon dioxide concentration are plotted against level of water availability. A comparison of Figs. 5 and 6 shows that similar effects have been produced on both sets of processes, with one important exception. Whereas in air the level of water availability has a considerable effect on dry-weight increase, it has no effect on either leaf length or coleoptile length. With high concentrations of carbon dioxide both leaf and coleoptile length and dry weight rise and then fall with decreasing water availability. At intermediate levels of water availability high concentrations of carbon dioxide tend to increase coleoptile length. With first leaves the effect of 20 per cent. carbon dioxide is doubtful; 10 per cent. carbon dioxide undoubtedly depresses linear growth. At extreme levels of water availability high concentrations of carbon dioxide consistently depress both coleoptile length and first leaf length.

TABLE V

Effect of Water Availability and Concentration of Carbon Dioxide on Water Content. Water Content as Percentage of Dry Weight

Percentage gelatine content of substrate. Percentage concentration of CO$_2$ / Age of seedlings (hrs.)	0 (water)			10			20			30		
	0·03	10	20	0·03	10	20	0·03	10	20	0·03	10	20
24	310	257	340	350	320	360	260	370	390	320	300	320
48	700	660	..	600	640	710	530	720	740	680	630	770
72	920	810	870	930	900	1,010	800	920	980	935	920	910
96	1,030	990	1,030	1,130	990	1,130	970	1,050	1,150	1,110	1,170	970

Water content during growth is evidently unaffected by either the level of water availability or by the concentration of carbon dioxide during the pre-treatment period.

A comparison of the results of Fig. 4 with the comparable data of Fig. 3*a* provides some indication of the extent to which injury to the embryo during excision affects the subsequent growth of the seedling. The embryos for the experiment of Fig. 4 were excised at 2 hours. One series pretreated with 10 per cent. gelatine and 20 per cent. carbon dioxide reached an average dry weight per embryo of 3·86 mg. This is only a little less than the comparable dry weight, 4·12 mg., reached at 96 hours by seedlings excised at 8 hours (Fig. 3*a*); this suggests that the large difference between the series excised at 2 hours and 6 hours (Fig. 3*a*) is not due to injury to the earlier excised seedlings.

The effect of the concentration of oxygen during pretreatment. The effect of various partial pressures of oxygen was examined with 10 per cent. gelatine as substrate. These experiments involved five series each exposed to a different gas mixture during pretreatment. The gas mixtures used contained 0, 5, 10,
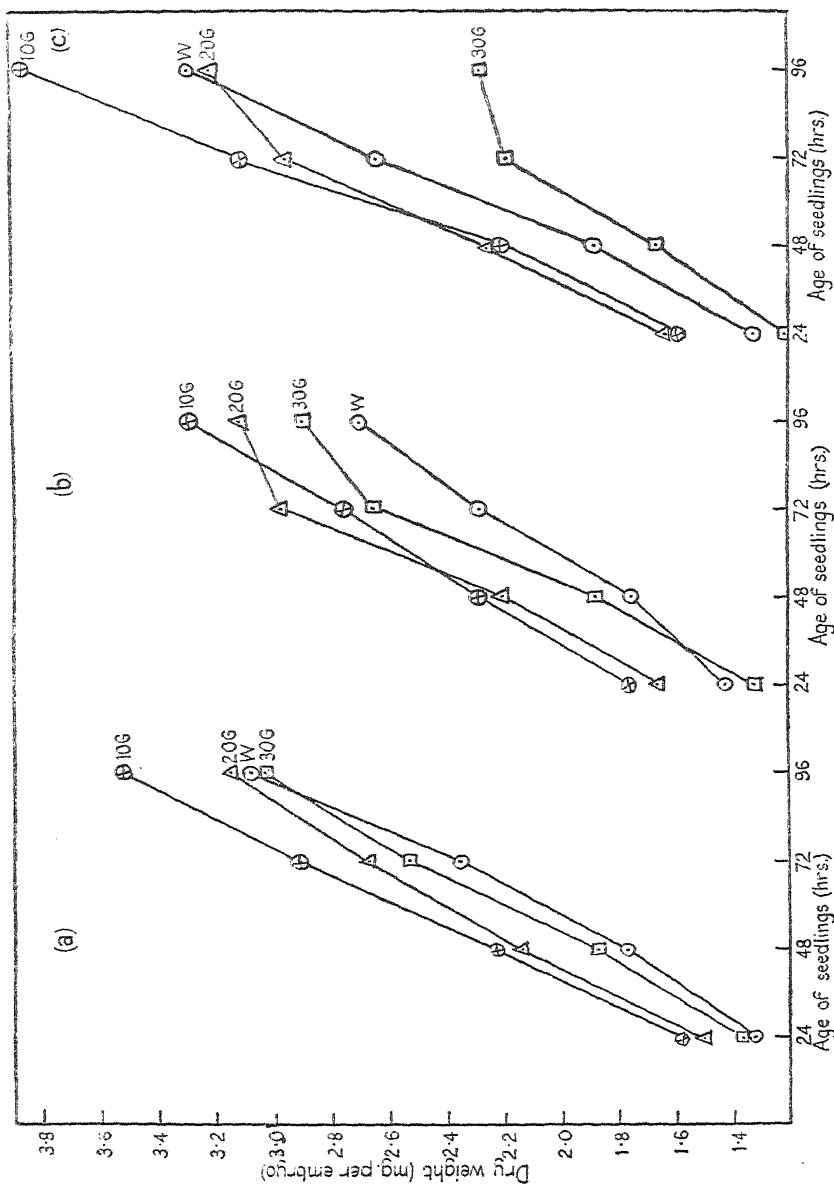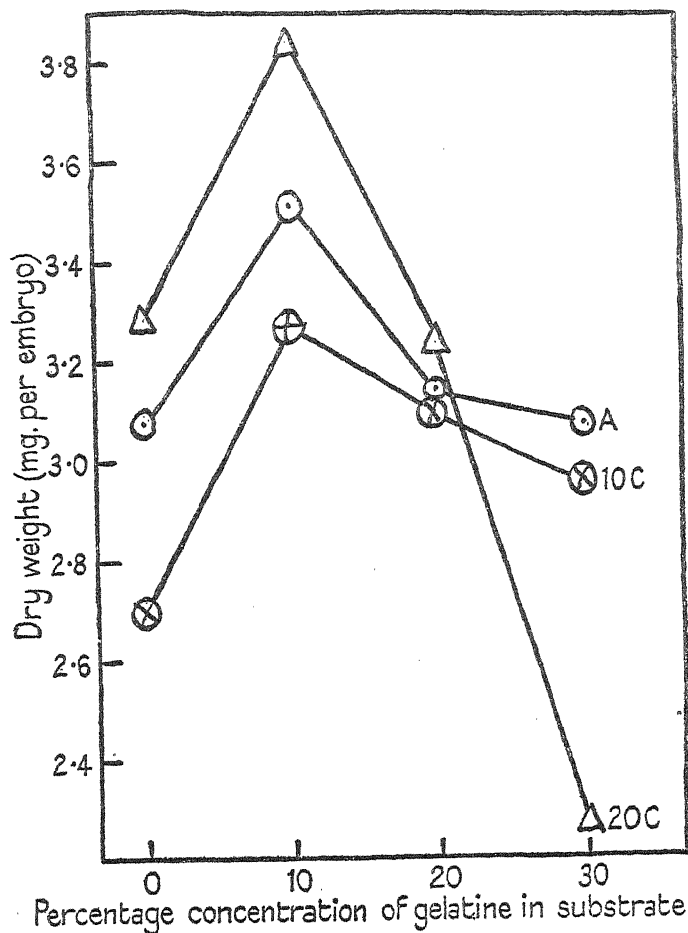

FIG. 6. Effect of level of water availability in substrate and of carbon dioxide in atmosphere during pretreatment on average dry weight of seedlings of final sample taken at 96 hours. Percentage concentration of gelatine along abscissae: 0 indicates water; A indicates air, 10 c indicates 10 per cent. carbon dioxide, and 20 c indicates 20 per cent. of carbon dioxide.

15, and 20 per cent. oxygen. Fig. 7 shows the subsequent dry weights recorded in each series. Table VI gives the length of the coleoptiles and leaves of the final samples of each series. Table VII gives the water contents corresponding to the data of Fig. 7.

The partial pressure of oxygen applied during the pretreatment period evidently has a considerable effect on the subsequent rate of dry weight increase. The corresponding values for the first three samples exposed to 20

per cent. and 15 per cent. oxygen do not differ significantly, but the final values show a considerable difference in favour of the series exposed to air. Groups treated with gas mixtures having a percentage concentration of oxygen less than 15 per cent. give very much lower rates of dry weight increase.
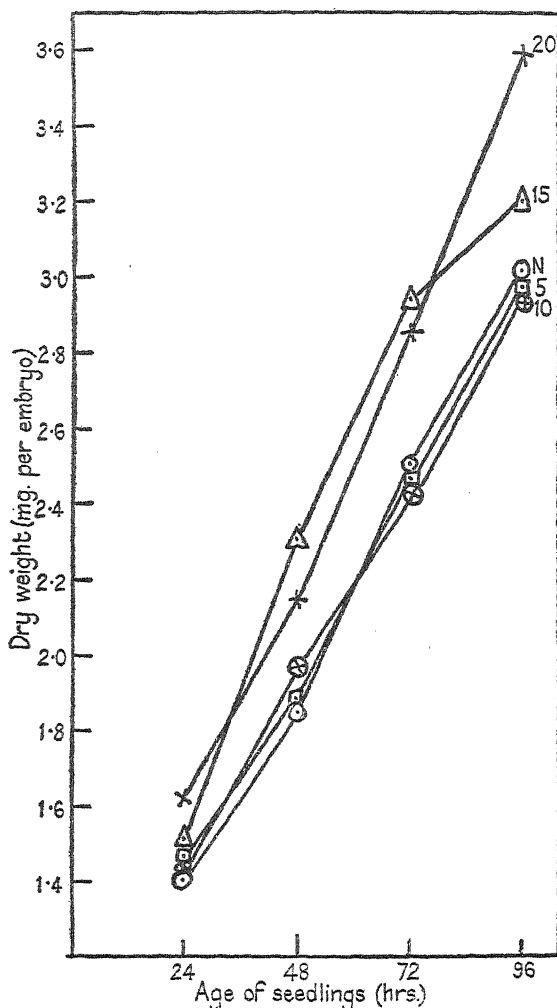
FIG. 7. Effect of percentage concentration of oxygen in atmosphere during pretreatment on subsequent growth in dry weight: N indicates pure nitrogen; figures attached to other curves indicate percentage composition of O_2.

There are, however, no differences between the corresponding samples of series exposed to atmospheres containing 0, 5, and 10 per cent. oxygen.

From Table VI it is evident that the lengths of both coleoptiles and first leaves are only very slightly affected by the partial pressure of oxygen during pretreatment. There is a slight increase with both organs as the concentration of oxygen is increased from 15 to 20 per cent.

TABLE VI

Effect of Partial Pressure of Oxygen during Pretreatment on Average Coleoptile and First Leaf Length (cm.) of Seedlings in Final Samples taken at 96 Hours

Concentration of O_2. (%).	Coleoptile.	First leaf.
0	2·03	1·20
5	2·11	1·36
10	1·85	1·05
15	2·12	1·23
20	2·50	1·66

TABLE VII

Effect on Water Content of Partial Pressure of O_2 during Pretreatment. Water Contents as Percentage of Dry Weight

Concentration of O_2 (%).	0	5	10	15	20
Age of seedlings (hrs.) 24	362	283	328	370	312
48	713	701	610	735	710
72	990	1,030 (?)	800	890	970
96	970	989	970	1,010	1,100

As in the previous set of experiments pretreatment has apparently little or no effect on the subsequent water content established in the seedlings.

The two sets of experiments on the effects of carbon dioxide and oxygen each incorporate a series in which during pretreatment the substrate is 10 per cent. gelatine and the atmosphere is air, a comparison of the results of which provides a further indication of the adequacy of the technique. The dry weights (mg.) in oxygen given in the two duplicate experiments are at 24 hours 1·56 and 1·62, at 48 hours 2·22 and 2·15, at 72 hours 2·90 and 2·85, and at 96 hours 3·52 and 3·59; the coleoptile lengths (cm.) at 96 hours are 2·5 and 2·66; and the first leaf lengths at 96 hours are 1·57 and 1·66.

Effect of nutrient in the substrate during pretreatment. The effect of the presence of the nutrient in the substrate to which the embryos are transferred is given by a comparison of the results of two experiments, each taken from one of two different series. Fig. 3a and Tables III and IV show the effects of excision at 2 hours followed by immediate removal to the nutrient medium; Figs. 4 and 5 and Table V show the effects of excising the embryo at 2 hours and then transferring to water for 6 hours before placing it in the culture medium. The water content and dry weight data of the two series are reproduced in Table VIII and the coleoptile and first leaf lengths data in Table IX.

The presence of the nutrient has little effect on the final dry weight, but the course of the dry weight change with time is different in the two series. When the nutrient is present, there is an initial phase of rapid increase, followed by another in which the rate of increase is lower. The water content during growth is slightly higher when the nutrient has been applied immediately after excision.

TABLE VIII

Effect of Nutrient during Pretreatment on Subsequent Dry Weight (mg. per Embryo) and Water Content (Percentage of Dry Weight)

Pretreatment substrate.	Dry weight. Water.	Nutrient.	Water content. Water.	Nutrient.
Age of seedlings (hrs.) ⎰24	1·32	1·25	310	515
48	1·76	2·20	700	692
72	2·35	2·25	920	1,190
96	3·08	3·02	1,030	1,122

TABLE IX

Effect of Nutrient during Pretreatment on Average Length of Coleoptile and First Leaf (cm.) of Seedlings in Final Samples taken at 96 Hours

Pretreatment substrate		Coleoptile.	First leaf.
	Water	2·57	1·56
	Nutrient	2·10	1·05

Clearly, as compared with the effect of water, that of the nutrient is to depress the linear growth of both the coleoptile and the first leaf.

DISCUSSION OF RESULTS

Of the aspects of growth measured in this investigation the water content is distinguished from length and dry weight by being uninfluenced by pretreatment. With the doubtful exception of nutrient level (Table VIII) all other factors applied during pretreatment have no effect on the percentage water content. The significance of this depends on the character of the growth process involved. The seedling in the dormant state consists of a dense mass of non-vacuolated cells, and early development is characterized by the vacuolation and consequent extension of these. Thus as development continues the water content increases, and while it is still increasing it may be supposed that the full extension of all cells induced by vacuolation has not been achieved. In the present series of experiments the water content continues to increase at least until the seedlings are 72 hours old, and it is therefore probable that the full transformation of the seedling from the dormant to the fully active state occupies 72 hours. Further, since the degree of vacuolation must affect profoundly the water content, the fact that, with one doubtful exception, the water content at 72 hours remains constant suggests that the rate at which the transformation from the dormant to the active state occurs is not affected by pretreatment.

The effect of pretreatment on linear growth of coleoptiles and leaves and on dry weight is far otherwise. On these pretreatment has a profound effect. Although the length measurements describe the linear growth of certain organs, they probably provide an indication of the linear growth of all the tissues of the seedling. Avery and Burkholder (1936) have shown that the increasing size of the coleoptile after it has reached 1 cm. is due almost entirely to cell extension. It is improbable that the conditions that determine the

extension of this organ do not also condition the extension growth of cells in other tissues, and the linear growth of this organ may therefore be taken as an indication of the effect of pretreatment on the linear growth of all the tissues of the embryo. The dry weight, of course, is determined on the whole seedling. Thus the results of pretreatment on the dry weight growth and linear growth of certain organs both undoubtedly indicate effects on cellular extension and on the deposition of dry matter in the growing cells of all tissues.

The data presented above showing the effect of time of excision on subsequent growth are in agreement with those of certain investigators who used different species. But the results of the present investigation show that the time of excision within the first 12 hours of germination affects not only the linear growth but also the dry weight growth of the whole seedling.

Schander (1934) and De Ropp (1939) have attributed the stimulating effect of the length of the period of attachment to the absorption of accessory food factors by the embryo during that time. These two investigators used cereal grains. Brunner (1932), using the seed of *Pinus maritima*, which is similar to that of the Gramineae in having an endosperm, found that seedlings isolated at 48 hours grew more vigorously than others isolated 24 hours earlier, and he suggested that the effect was due to the greater absorption of hormone over the longer period of attachment. The time-intervals involved in the experiment of Brunner are much longer than in those of Schander and De Ropp, but the two sets of observations may refer to the same phenomenon, since the rate of germination in the pine is very much lower than it is in the gramineous seed. Cholodny (1935), in the endosperm of maize, found a growth hormone which he identified as blastanin and showed that it was progressively absorbed by the seedling during the first 48 hours of germination. Pohl (1936), using Avena, claims to have shown that the growth of the seedling is reduced by the electrolytic removal from the endosperm of the active auxin ion. Avery, Creighton, and Shalucha (1940) have demonstrated the presence of auxin in the endosperm of maize, and they have also shown that it decreases during germination. The direct evidence for an absorption of hormone during the first 6–8 hours of germination is, however, not available. Nevertheless, since it is present in the endosperm (although Hatcher and Gregory (1941) have recently shown that the auxin content of barley endosperm is comparatively low), it is not improbable that an absorption of hormone during this early period does in fact occur. Further, it is probable that such an absorption contributes to the development of the effects observed, but it is clear that it cannot be the only operative factor. The incident levels of certain environmental factors in the seed and in the culture medium are undoubtedly also involved.

The reserve food materials of the endosperm are not available to the seedling during the first 24 hours of germination. Thus embryos excised 8 hours after water uptake begins are not in contact during this time with a solution containing soluble carbohydrates and considerable quantities of mineral salts, but these are the conditions to which the seedling is exposed for 6 of these 8 hours when it is excised at 2 hours and transferred immediately to the culture

solution. Now the results presented in Table IX indicate that the effect of the nutrient medium during the critical 6-hour period is that of depressing the extension growth of the seedling. Thus the more vigorous growth in length of the coleoptile and of the first leaf of seedlings excised at 8 hours is likely to be due in part to the depressing effect of the culture solution on the seedlings excised at 2 hours, established during a period when the later excised seedlings are not exposed to the same conditions. Table VIII suggests, on the other hand, that the solute content of the substrate has little influence on dry-weight growth.

It has been shown (Brown, 1943) that the internal concentration of carbon dioxide during the first 12 hours of germination corresponds to an average partial pressure of 0·1 atm., and that during certain periods it exceeds this value. With water and 10 per cent. gelatine—this substrate being one which reproduces closely the condition of water availability in the seed— the effect of increasing the concentration of carbon dioxide is first to depress and then to increase both dry weight (Fig. 4) and the length of the coleoptile and of the first leaf (Fig. 5). With 20 per cent. carbon dioxide the growth made is greater than it is with air (Fig. 4). Now as the period of attachment is increased to 6 hours, both dry weight and linear growth increase. During this period the internal concentration of CO_2 may approach 20 per cent., and it is possible that the stimulation is in fact due to this factor; but the observed effect of lower concentrations of carbon dioxide places a serious limitation on this interpretation. It must be emphasized, however, that the carbon-dioxide effect may not be due to the carbon dioxide as such. The acidity of the pre-treatment substrate was probably altered by absorption of carbon dioxide, and Schander (1934) has shown that the acidity of the endosperm does in fact change during germination. It may therefore be that carbon dioxide both in the artificial environment and in the seed exerts an indirect effect by modifying the acidity of the substrate.

The level of water availability in the embryo in the seed is low. Eight hours after water uptake by the whole seed begins the water content of the embryo is about 120 per cent. Embryos excised at 2 hours and transferred to the culture solution immediately, reach a water content on the other hand of about 160 per cent. within a further 2 hours. The water contents reached on the substrate media used in these investigations are therefore of some significance. The water content after 8 hours' exposure to water is 180 per cent.; to 10 per cent. gelatine, 135 per cent.; to 20 per cent. gelatine, 114 per cent.; and to 30 per cent. gelatine, 91 per cent. The conditions of the seed for water uptake are therefore most closely reproduced by the 10 and 20 per cent. gelatine media. It is these media which give the highest subsequent dry weight (Fig. 4). Attachment to the seed also promotes both dry weight and linear growth. Thus it may be assumed with some confidence that the low level of water availability establishes a condition which stimulates the dry weight growth of the embryo.

Although dry weight is markedly affected by the level of water availability during pretreatment, the lengths of the coleoptile and of the first leaf are only

affected by the level of water availability when the concentration of carbon dioxide is high (Fig. 5). As stated above, the concentration of carbon dioxide inside the seed is never low. The later excised seedlings sustain a more vigorous extension growth than those excised earlier, and it is therefore probable that the level of water availability inside the seed stimulates not only dry weight but also extension growth.

Each of the factors considered above tends to be at a level inside the seed which, relative to the incident level of the same factor outside the seed, stimulates the subsequent growth of the seedling. The data of Fig. 7 and Table VI suggest, on the other hand, that the change in the oxygen concentration consequent on excision is not such as to influence greatly the subsequent growth of the seedling. It would appear that both dry weight and extension growth are not seriously affected until the oxygen concentration is reduced below 15 per cent. An estimate of the internal concentration of oxygen based on gaseous exchange data for the whole seed gave an average value of about 10 per cent., but at certain times the value must be higher, and it may therefore be suggested that the growth of the seedling is not affected by the change from an atmosphere having an average partial pressure of 10 per cent. to one containing 20 per cent. oxygen.

It is clear from the data of Fig. 3*a* and Table III that after a certain critical period has been reached further extension of the period of attachment exerts a depressing effect on the growth of the seedling. De Ropp (1939) has reported a similar effect. The present data provide no indication of the significance of this observation. It is, however, probable that the stimulation exerted by each of the factors enumerated above is a function of the time of exposure which in turn probably varies with the incident level of each factor during exposure.

The effects of extreme levels of carbon dioxide, oxygen, and water that do not correspond to conditions in the seed are given here to indicate the limits within which the levels of each factor must be established in the seed to develop the effects required by the observations on the influence of the period of attachment on the subsequent growth rate. The whole range of effects, considered in relation to the time and the phase of development in which they are established, have, however, another significance. The results reported here all show that the particular conditions to which the embryo is exposed during the comparatively short period of 6 hours immediately after active development begins have a profound effect on the subsequent growth of the seedling. This suggests a high degree of instability in the metabolic pattern of the embryo, the stabilization of the metabolic state being subject to modification according to the nature of the environment in which early development occurs.

SUMMARY

1. A method for culturing isolated embryos in a non-sterile condition is described.

2. The effect of the length of time, up to 10 hours, during which the

embryo is attached to the seed before it is excised is investigated. It is shown that the subsequent growth of the seedling tends to increase as the time of excision is increased from 2 to 6 hours and that it tends to decrease as the time of excision is still further increased from 6 to 10 hours.

3. The origin of the effect of time of excision is investigated by determining the independent effects on subsequent growth of exposing embryos, excised from seeds 2 hours after water uptake begins, to different partial pressures of oxygen and carbon dioxide, and to different levels of water availability for a further period of 6 hours, before they are transferred to a nutrient medium. The effect of the presence of the nutrient during this 6-hour pretreatment period was also investigated.

4. The subsequent growth of the embryo is profoundly affected by the conditions to which it is exposed for 6 hours after excision at 2 hours.

5. The data of this together with others from another investigation show that the conditions after removal from the seed at 2 hours and exposure to a culture medium are less favourable for subsequent growth in respect of the incident levels of nutrient, water availability, and possibly carbon dioxide, than they are in the seed. The effect of the different partial pressure of oxygen to which the seedling is exposed after excision is probably negligible.

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Studies in Tropical Fruits

XV. Hemicellulose Metabolism of the Banana Fruit during Storage and Ripening

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With twelve Figures in the Text

INTRODUCTION

IN previous studies on the carbohydrate metabolism of the banana fruit (Barnell, 1941, 1941*a*, 1942) a considerable amount of unidentified material was found to be present in both pulp and skin, the bulk of it being in the non-starch fraction of the alcohol-insoluble residue. It decreased during the ripening of the fruit, particularly in the pulp. The non-starch fraction appeared too large, especially in the pulps of unripe fruits, to be entirely cellulose; accordingly the possibility that it contained hemicelluloses and pectins was considered.

Hexoses after condensation to hexosans may have their free primary alcoholic groups oxidized to form uronic anhydride units; decarboxylation will then give rise to pentosan units. A single hexosan, even if formed from only one hexose, can give rise to a large number of intermediate products. Of these products the pectins are unusually highly oxidized hexosans with little of the parent hexose and little of the final pentose in their constitutions. The remainder of the intermediates are classified as hemicelluloses.

The chemistry of this hemicellulose group still remains somewhat obscure as the individual substances have no very distinctive common property. They are usually defined as carbohydrate substances not removed from plant tissues by water but extracted by dilute (4 per cent.) alkali. Certain substances

of undoubted hemicellulosic nature are excluded by this definition, e.g. the dilute acid hydrolysable hemicelluloses of the apple (Widdowson, 1932), the water-soluble galactans of wood (Schorger and Smith, 1916), and the 'cellulosan' of Hawley and Norman (1932).

Surveys of the hemicellulose components of various plants have been made, particularly of woods (O'Dwyer, 1923, 1926, 1928, &c.; Preece, 1930) and of grasses (Buston, 1934, 1935). In these surveys the extracted hemicellulose was divided into various fractions and the hydrolysis products, hexose and pentose sugars and uronic acids, identified and estimated. In most instances the individual hemicelluloses were isolated.

Buston (1935) showed that in starving leaves or in leaves subjected to slow drying hemicellulose changed significantly, while no appreciable variation was observed in the amount of pectin. He concluded that hemicelluloses were more labile than pectins and that it is possible that they may be available for respiration under certain circumstances.

Murneek (1929) suggested that hemicelluloses were important storage carbohydrates in the woody parts and fruits of the apple. However, Widdowson (1932) observed that both hemicelluloses and pectin in the developing apple fruit increased steadily to a constant value which did not fall to any extent during subsequent storage of the fruit. She suggested from this that hemicelluloses do not serve as a reserve carbohydrate supply in the apple fruit.

The present work demonstrates the quantitative importance of hemicelluloses in the metabolism of the banana fruit. No attempt has been made either to isolate or to identify individual hemicelluloses. This is left as a fertile field for future investigation.

Since the value of a storage method depends on the ultimate quality of the product stored, i.e. on its chemical composition and physical texture, the relative proportions of hemicellulose, starch, and sugars in the eating-ripe banana are of considerable importance. It will be shown in the present work that the effects of storage at a low temperature on the rates of hydrolysis of starch and hemicellulose in banana pulp are very different and hence the composition of the pulp of a banana after different lengths of cold storage differs in respect of these two constituents at least.

MATERIAL AND METHODS

The material used in these investigations consisted of the residues, after alcohol extraction, from previous investigations of the carbohydrates of banana fruits (Gros Michel variety) during refrigerated storage and ripening (Barnell, 1941a, 1942). These studies were directed towards establishing the trends of the various major carbohydrates in the fruits during storage at 53° F., relative humidity 80–85 per cent. (i) for periods within the tolerance of the fruit, (ii) for periods which would produce symptoms of 'chilling'[1] and,

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[1] See Barnell (1941a) for a description of 'chilling' of bananas.

for (i) and (ii), during the subsequent period of ripening at 68° F. with relative humidity approximately 70 per cent., (iii) continuously at 53° F. to produce intensive chilling effects.

For (i) and (ii) lots of 30 bunches each of standard '¾-full' fruit and 'heavy ¾-full'[1] fruit were obtained from the Toco district of Trinidad. One lot of each grade of fruit received a 'short' period of cold storage and one lot a 'long' period. For the standard ¾-full fruit a short period was 14 days and a long period 20 days; for the heavy ¾-full fruit the periods were 7 and 14 days respectively. For (iii) 30 bunches of heavy ¾-full bananas were used.

A full description of the behaviour of the fruit has already been given (Barnell, 1941a, 1942): it will suffice here to say that the short-period fruit all ripened normally while most of the long-period fruit showed symptoms of chilling; the fruit stored continuously at 53° F. was intensively chilled.

The dried alcohol-extracted residues were stored in glass tubes closed with waxed corks until required for the present investigation.

The methods adopted for the estimation of pectins, cellulose, and hemicelluloses closely followed those of Widdowson (1932); they consisted in determining the proportion of the residue insoluble in $N/75$ hydrochloric acid and the proportion of pectin present in the acid extract. One-gramme samples of each of the dried alcohol-insoluble residues (in duplicate) were refluxed for 3 hours with 100 ml. $N/75$ acid; the acid was then poured through a filter-paper of known dry weight or a Gooch crucible and the residue refluxed with a further 100 ml. of acid for a further 3 hours. The second volume of acid together with the remaining residue was then filtered through the original filter-paper or crucible, and the residue washed with a little $N/75$ hydrochloric acid. Refluxing with a third 100 ml. of acid for 3 hours did not appreciably increase the pectin extracted, so in routine estimations it was omitted.

The residue was dried at 100° C. and weighed; this fraction was termed 'cellulose', while the filtrate contained pectins, starch, and hemicellulose hydrolysates extracted by the dilute acid.

The filtrate was neutralized with dilute caustic soda and the precipitation of pectin, as calcium pectate, carried out as described by Carré and Haynes (1922). The final filtration was through a Gooch crucible and the precipitate was dried to constant weight at 100° C. This gave the pectin present in the residue in terms of calcium pectate.

The cellulose and pectate values were converted to percentages of the original fresh weight of pulp and skin; the total alcohol-insoluble residue and the starch had previously been determined as percentages of the fresh weight (Barnell, 1941a, 1942). The quantity obtained by the difference (total alcohol-insoluble residue percentage of the fresh weight) *minus* (starch+ cellulose+pectin percentages) was regarded as the percentage of 'hemicellulose' in the fresh tissue.

[1] See Wardlaw, Leonard, and Barnell (1939) for explanation of terms.

Amounts of Pectic Substances in the Pulp and in the Skin

The estimation of the pectin present in the alcohol-insoluble residues was an integral part of the hemicellulose determination in pulp and skin. The pectin so estimated was that fraction of the total pectin remaining after
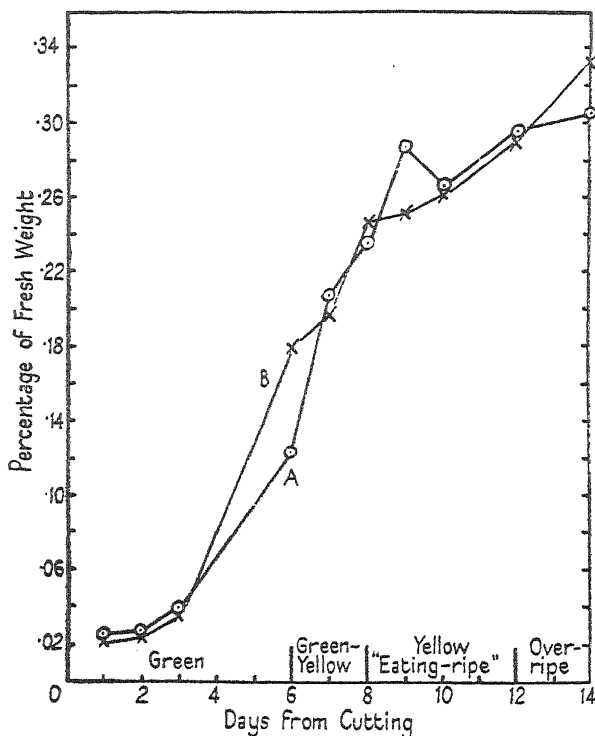
Fig. 1. Pulp. Soluble pectin (as calcium pectate) as percentage of the fresh weight. Data from two 'heavy ¾-full' bunches, *A* and *B*, ripening at tropical temperatures.

extraction of the fresh tissue by 80 per cent. alcohol in a soxhlet during a 6-hour extraction period. It therefore does not provide data of the changes in total soluble pectin in the banana.

Some preliminary data on the changes in percentage amounts of water-soluble pectin in the banana pulp during ripening under tropical temperatures have therefore been obtained and will be briefly discussed here. It is proposed later to extend these investigations, so the data presented will be limited to a demonstration that well-marked changes occur in the amount of soluble pectin in the pulp during ripening, though the amount present is never large.

Soluble pectin was extracted from the pulps by leaving the finely sliced pulps overnight in water at room temperature (approximately 80° F.). Fig. 1 sets out the soluble pectin (calcium pectate) percentages in fingers from the upper rows of the third and fourth hands of two heavy ¾-full

bunches (*A* and *B*) ripening under tropical conditions; the data for the fifth day are very low (0·013) and have been omitted.
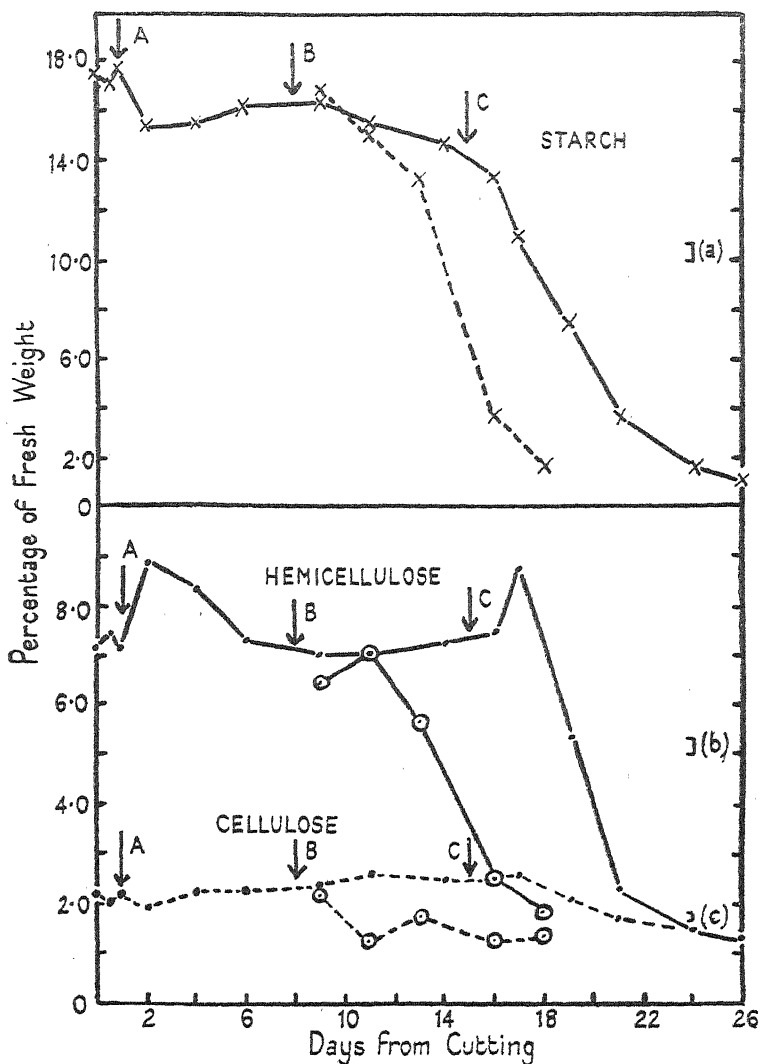
FIG. 2. Pulp. Percentage amounts of starch, hemicellulose, and cellulose in the pulps of heavy ¾-full bananas during storage at 53° F. and ripening at 68° F. The vertical arrows *A* indicate the time of placing the fruit in store, those labelled *B* and *C* the end of the storage period for short- and for long-storage fruits. The vertical lines (*a*), (*b*), and (*c*) represent the minimum significant differences (*P* = 0·05) of (*a*) starch, (*b*) hemicellulose, and (*c*) cellulose.

The short-storage (7-day) fruit remained green till day 10, yellowing occurred till day 14, after which the fruit was 'eating-ripe' till day 18. The corresponding stages for the long-storage (14-day) fruit were green till day 14, yellowing to day 19, ripe from day 19 to day 23, afterwards over-ripe.

Soluble pectin was present in almost negligible amount during the first few days after cutting, but increased relatively rapidly about the time the

fruit attained the 'sprung' condition[1] and while the colour was changing from green to yellow. During the eating-ripe and over-ripe stages the rate of increase was less.

The amounts of pectin (as calcium pectate), expressed as percentages of the fresh weight, in the residues from the pulps and skins of fruits receiving the various storage treatments are set out in Tables I, II, V, and VI for heavy ¾-full and in Tables III and IV for standard ¾-full fruit.

It will be observed that in the pulps of both grades of fruit the pectin left in the alcohol-extracted residue was small in amount and showed no definite trend. There was, on the average, four times as much pectin percentage of the fresh weight in the alcohol-extracted residue from the skin as from the pulp, but no definite indication of a trend in its percentage amount during storage and ripening.

Amounts of Hemicellulose and Cellulose in the Pulp

The data for hemicellulose and cellulose as percentages of the fresh weight of the pulp are set out in Tables I and III for heavy ¾-full fruit and standard

Table I

Composition (% of fresh weight) of the Pulp of heavy ¾-full Fruit during Storage at 53° F. and Ripening at 68° F.

Days from harvesting.	Temperature ° F.	Total alcohol-insoluble residue.	Starch.	Cellulose (insoluble in N/75 HCl).	Pectin (as calcium pectate).	Hemi-cellu-lose.	Starch plus Hemi-cellulose.
0	Room temp. (80°–85°)	26·76	17·45	2·21	0·00	7·10	24·55
16 hr.	,,	26·46	16·90	2·03	0·08	7·45	24·35
23 hr.	,,	26·87	17·65	2·14	0·00	7·08	24·73
2	53°	26·25	15·25	1·94	0·12	8·94	24·19
4	,,	26·18	15·53	2·29	0·00	8·36	23·89
6	,,	25·89	16·17	2·27	0·11	7·34	23·51
9	,,	25·68	16·30	2·36	0·00	7·02	23·32
11	,,	25·07	15·43	2·60	0·01	7·03	23·46
14	,,	24·50	14·67	2·53	0·00	7·30	21·97
16	68°	23·48	13·35	2·51	0·14	7·48	21·04
17	,,	22·48	10·91	2·60	0·14	8·83	19·74
19	,,	15·12	7·52	2·11	0·10	5·39	12·91
21	,,	7·97	3·67	1·74	0·26	2·30	5·97
24	,,	4·86	1·62	1·52	0·19	1·53	3·15
26	,,	4·01	1·13	1·38	0·16	1·34	2·47
9	68°	25·44	16·41	2·18	0·40	6·45	22·86
11	,,	23·51	15·03	1·23	0·19	7·06	22·09
13	,,	20·75	13·22	1·73	0·13	5·67	18·89
16	,,	7·69	3·74	1·25	0·19	2·51	6·25
18	,,	5·12	1·71	1·34	0·20	1·87	3·58
Significant difference:		—	0·65	0·23	—	0·71	—

¾-full fruit respectively during short and long storage, and in Table V for heavy ¾-full fruit stored continuously at 53° F. The starch content as percen-

[1] See Barnell, 1941, p. 271.

tage of the fresh weight has also been reproduced in the tables and figures to facilitate reference to the changes in other carbohydrates as given earlier (Barnell, 1941*a*, 1942).

In general the percentage hemicellulose content of the banana pulp followed, at a lower level except in over-ripe fruit, a course similar to that of starch.

TABLE II

Composition (% of fresh weight) of the Skin of heavy ¾-full Fruit during Storage at 53° F. and Ripening at 68° F.

Days from harvesting.	Temperature ° F.	Total alcohol-insoluble residue.	Starch.	Cellulose (insoluble in N/75 HCl).	Pectin (as calcium pectate).	Hemi-cellu-lose.	Starch plus Hemi-cellulose.
0	Room temp. (80°–85°)	9·97	3·75	2·97	0·48	2·77	6·52
16 hr.	,,	9·91	3·57	2·86	0·42	3·06	6·63
23 hr.	,,	10·12	3·46	2·96	0·19	3·50	6·96
2	53° F.	9·42	2·79	2·90	0·38	3·35	6·14
4	,,	9·56	2·36	3·36	0·41	3·43	5·79
6	,,	9·61	2·03	3·14	0·47	3·97	6·00
9	,,	9·43	2·48	2·98	0·53	3·44	5·92
11	,,	9·70	2·51	3·29	0·36	3·54	6·05
14	,,	9·60	2·39	3·53	0·44	3·24	5·63
16	68°.	9·64	2·19	3·84	0·49	3·12	5·31
17	,,	9·74	2·34	3·86	0·56	2·98	5·32
19	,,	9·04	1·74	4·13	0·59	2·58	4·32
21	,,	8·66	0·62	4·36	0·43	3·25	3·87
24	,,	8·86	0·29	4·89	0·62	3·06	3·35
26	,,	10·36	0·48	5·79	0·61	3·48	3·96
9	68°	9·33	2·65	3·14	0·51	3·03	5·68
11	,,	9·16	2·45	3·42	0·51	2·78	5·23
13	,,	8·23	1·61	3·34	0·55	2·73	4·34
16	,,	6·90	0·47	3·65	0·47	2·31	2·78
18	,,	7·38	0·29	3·97	0·60	2·52	2·81
Significant difference:		—	0·33	0·24	—	0·37	—

It did not show any large change in amount whilst the fruit was still green, but decreased relatively rapidly during the subsequent ripening to a new low level.

A closer examination of the trends of the hemicellulose and starch percentages during the first few days of refrigerated storage (Figs. 2, 4, and 6) suggests that they are, to some extent, complementary. The sums of the percentages of starch and hemicelluloses are given in column 8 of Tables I and III and in column 7 of Table V and are plotted for the short- and long-stored heavy ¾-full grade of fruit in Fig. 7; very smooth curves are obtained. The summated fractions fell slowly and steadily from the time of cutting for the long-storage fruit until the 11th day, after which the rate of fall increased; it became very much greater after the fruit was placed in the ripening room. For the short-storage fruit the increased rate of fall occurred immediately after the fruit was in the ripening room.

In the standard ¾-full fruit the one anomalous high value for the sum of

starch and hemicellulose observed on the third day (column 8 of Table III)
is easily within the limit required for significant difference.[1] Although it
is not impossible that the small decrease in starch percentage observed in

Fig. 3. Skin. Percentage amounts of starch, hemicellulose, and cellulose in the skins of
heavy ¾-full bananas during storage at 53° F. and ripening at 68° F. The arrows *A*, *B*, *C* and
vertical lines (*a*), (*b*), (*c*) have the same significance as in Fig. 2.
   The ripening record for this fruit is given in the subscript to Fig. 2.

the pulp during the first few days of storage and the simultaneous increase
in hemicellulose (estimated by difference and involving the starch value)

   [1] Values for starch and hemicellulose significant differences are given at the foot of the
respective columns and as vertical lines in the figures.

may be due to irregularity in the taka-diastase estimation of starch, the effect has been observed over several determinations. It therefore appears probable that there is some conversion in the pulp first of starch to hemicelluloses

TABLE III

Composition (% of fresh weight) of the Pulp of standard ¾-full Fruit during Storage at 53° F. and Ripening at 68° F.

Days from harvesting.	Temperature ° F.	Total alcohol-insoluble residue.	Starch.	Cellulose (insoluble in N/75 HCl).	Pectin (as calcium pectate).	Hemi-cellu-lose.	Starch plus Hemi-cellulose.
0	Room temp. (80°–85°)	27·22	16·78	2·64	0·00	7·80	24·58
20 hr.	,,	27·13	15·96	2·62	0·06	8·49	24·45
26 hr.	53°	27·68	15·91	2·78	0·04	8·95	24·86
3	,,	28·18	15·66	2·13	0·09	10·30	25·96
6	,,	26·96	16·45	2·40	0·06	8·05	24·50
10	,,	27·04	16·99	2·53	0·09	7·43	24·42
14	,,	26·36	16·56	2·18	0·10	7·52	24·08
17	,,	25·25	16·26	2·23	0·11	6·65	22·91
19	,,	24·48	14·53	2·85	0·58	6·52	21·05
21	68°	22·68	13·34	2·42	0·02	6·90	20·24
23	,,	16·60	9·55	2·25	0·11	4·69	14·24
25	,,	10·29	5·49	2·06	0·41	2·33	7·82
27	,,	5·77	2·13	2·14	0·04	1·46	3·59
29	,,	4·85	1·51	1·49	0·10	1·75	3·26
31	,,	4·04	1·08	2·24	0·00	0·72	1·80
33	,,	3·36	0·70	1·67	0·00	0·99	1·69
16	68°	26·07	16·47	3·39	0·00	6·21	22·68
18	,,	19·12	12·25	1·93	0·00	4·94	17·19
20	,,	9·76	5·25	1·87	0·07	2·57	7·82
22	,,	6·80	2·89	1·68	0·04	2·19	5·08
24	,,	4·82	1·61	1·58	0·03	1·60	3·21
26	,,	3·84	0·88	1·38	0·01	1·57	2·45
28	,,	3·70	0·85	1·55	0·02	1·28	2·13
Significant difference :		—	0·67	0·37	—	0·78	—

and then of hemicelluloses back to starch during the early stages of storage of bananas.

In Fig. 6 the percentage of starch, hemicellulose, and cellulose are plotted from the data of Table V for the entire 99-days period during which the fruit was at 53° F.; the starch values do not show any definite trend during the first 20 days, but thereafter fall taking a total of approximately 40 days to reach the level associated with eating-ripe fruit. The hemicellulose percentage after an initial fall shows little change until the 25th day, after which it falls rapidly to low values by the 40th day. This latter behaviour is in contrast with that of starch in which, while there was a marked transition from low to high rate of fall occurring at approximately the same time as that for hemicellulose, the rate of fall was greatly slowed down by the temperature of storage.

It has previously been shown (Barnell, 1941*a*) that during the stage of

starch disappearance in the pulp there is a logarithmic relation between the starch percentage and time. An index, K_s, the slope of the log. starch percentage line, was used to demonstrate that the rate of starch disappearance

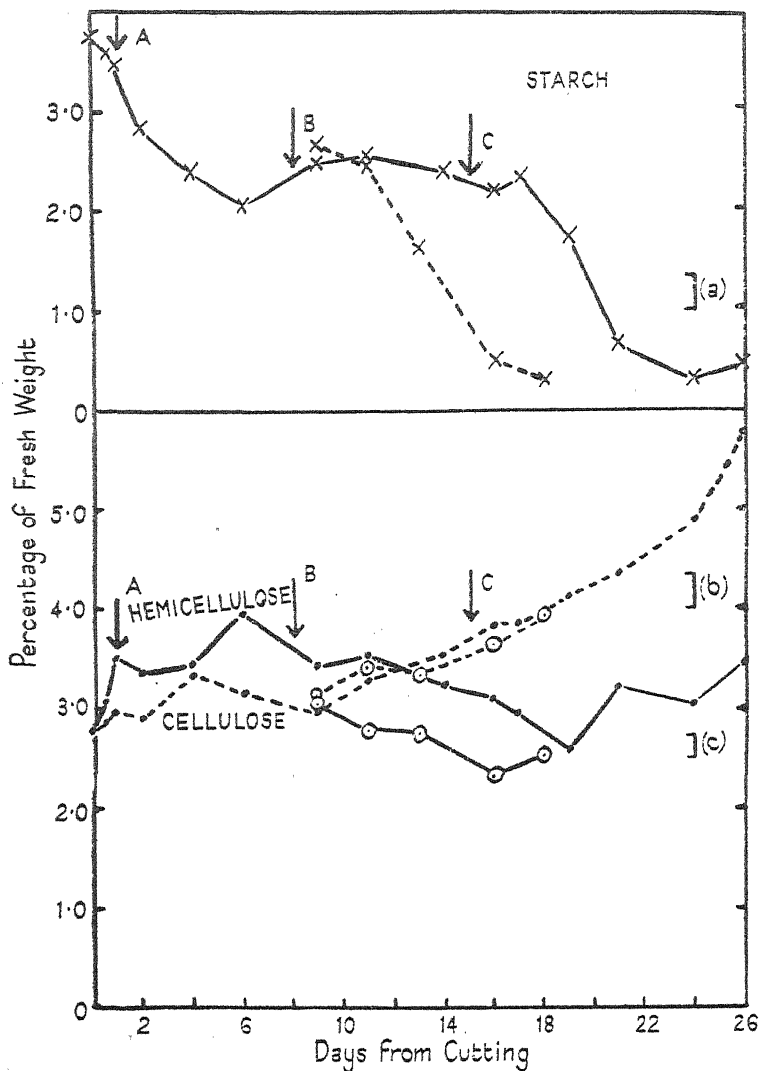

FIG. 4. Pulp. Percentage amounts of starch, hemicellulose, and cellulose in the pulps of $\frac{3}{4}$-full bananas during storage at 53° F. and ripening at 68° F. The arrows *A*, *B*, *C* and the vertical lines (*a*), (*b*), (*c*) have the same significance as in Fig. 2.

The short-storage (14-day) fruit remained green till day 15, yellowing occurred till day 19, after which the fruit was eating-ripe till day 23 and then became over-ripe. The corresponding stages for the long-storage (20-day) fruit were green till day 20, yellowing to day 24, ripe from day 24 to day 29, afterwards over-ripe.

at 68° F. was slower in long-stored, and therefore chilled, fruit than in short-storage fruit. In Fig. 8 the logarithms of the hemicellulose percentage for each set of data have been plotted against time for the stage in which hemicellulose was decreasing, while in Fig. 9 the corresponding starch values are plotted against the corresponding times. It will be observed that the hemicellulose values lie approximately on straight lines parallel with each other while the lines drawn through the sets of starch values are

TABLE IV

Composition (% of fresh weight) of the Skin of standard ¾-full Fruit during Storage at 53° F. and Ripening at 68° F.

Days from harvesting.	Temperature ° F.	Total alcohol-insoluble residue.	Starch.	Cellulose (insoluble in N/75 HCl).	Pectin (as calcium pectate).	Hemi-cellu-lose.	Starch plus Hemi-cellulose.
0	Room temp. (80°–85°)	9·69	3·20	3·04	0·37	3·08	6·28
20 hr.	,,	9·84	3·18	3·13	0·30	3·23	6·41
26 hr.	53°	9·77	2·94	3·14	0·40	3·29	6·23
3	,,	9·75	2·90	3·12	0·22	3·51	6·41
6	,,	9·83	2·86	3·49	0·24	3·24	6·10
10	,,	9·83	2·88	3·74	0·20	3·01	5·89
14	,,	9·53	2·96	3·86	0·91	1·80	4·76
17	,,	9·55	2·36	3·95	0·80	2·44	4·80
19	,,	9·95	2·33	4·04	0·30	3·28	5·61
21	68°	9·86	2·28	3·82	0·56	3·20	5·48
23	,,	9·74	2·12	4·64	0·60	2·38	4·50
25	,,	8·93	1·12	4·19	0·61	3·01	4·13
27	,,	9·52	0·51	5·20	0·57	3·24	3·75
29	,,	9·90	0·50	5·42	0·60	3·38	3·88
31	,,	10·32	0·44	5·70	0·50	3·68	4·12
33	,,	10·59	0·38	5·06	0·39	4·76	5·14
16	68°	9·60	2·40	3·20	0·35	3·65	6·05
18	,,	9·09	2·23	3·19	0·42	3·25	5·48
20	,,	8·08	0·84	4·02	0·40	2·82	3·66
22	,,	7·85	0·50	4·06	0·36	2·93	3·43
24	,,	8·24	0·46	4·47	0·35	2·96	3·42
26	,,	8·78	0·37	5·05	0·37	2·99	3·36
28	,,	9·39	0·40	4·99	0·39	3·61	4·01
Significant difference:		—	0·21	0·48	—	0·37	—

TABLE V

Composition (% of fresh weight) of the Pulp of heavy ¾-full Fruit during continuous Storage at 53° F.

Days from cutting.	Total alcohol-insoluble residue.	Starch.	Cellulose (insoluble in N/75 HCl).	Pectin (as calcium pectate).	Hemi-cellu-lose.	Starch plus Hemicellu-lose.
1	26·16	13·44	1·18	0·50	11·04	24·48
2	26·41	14·21	1·30	0·41	10·49	24·70
9	25·35	14·11	1·25	0·50	9·49	23·60
14	24·82	13·44	1·35	0·48	9·55	22·99
17	24·89	14·18	1·48	0·40	8·83	23·01
21	24·09	12·95	1·30	0·35	9·49	22·44
25	21·55	10·23	1·70	0·57	9·11	19·34
29	18·04	8·38	1·35	0·54	7·77	16·15
33	15·58	7·36	1·85	0·29	6·08	13·44
37	7·98	4·88	1·15	0·13	1·82	6·70
44	5·69	3·13	1·23	0·17	1·16	4·29
51	4·59	2·10	1·29	0·11	1·09	3·19
58	3·80	0·69	1·15	0·22	1·74	2·43
75	3·11	0·44	1·13	0·21	1·33	1·77
85	2·81	0·15	1·24	0·11	1·31	1·46
93	2·89	0·35	1·29	0·02	1·23	1·58
99	3·43	0·37	1·65	0·07	1·34	1·71
Significant difference:	—	0·589	0·292	—	0·731	—

divergent. Values for K_h, the hemicellulose index, have been obtained from the slopes of these lines for comparison with the values already obtained for K_s, the starch index. These values, which within the limits of the data are identical, are given in Table VII along with those for K_s.
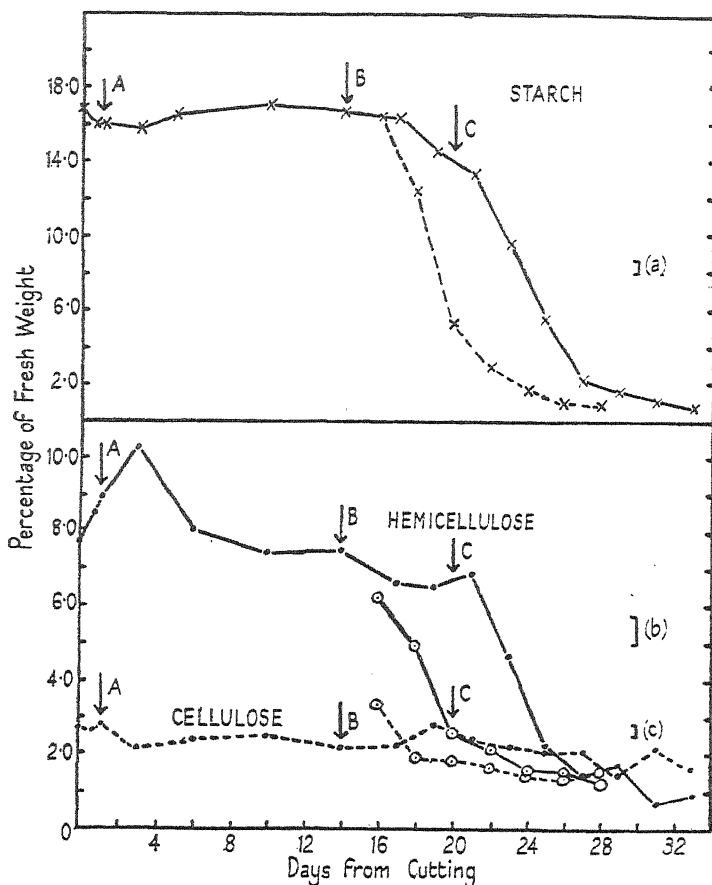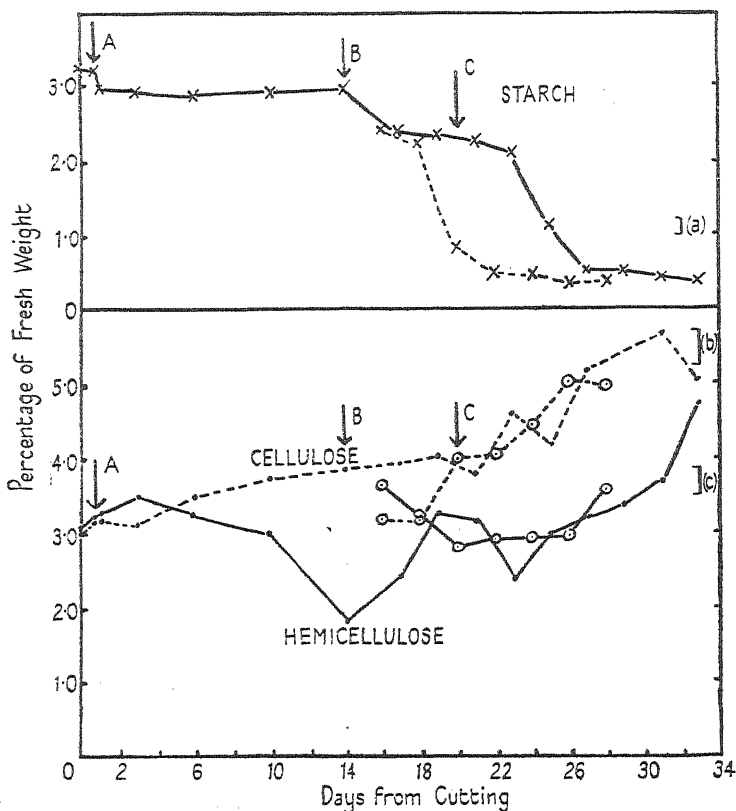

FIG. 5. Skin. Percentage amounts of starch, hemicellulose, and cellulose in the skins of ¾-full bananas during storage at 53° F. and ripening at 68° F. The arrows *A*, *B*, *C* and the vertical lines (*a*), (*b*), (*c*) have the same significance as in Fig. 2.
  The ripening record for the ¾-full fruit is given in the subscript to Fig. 4.

The table shows clearly that whereas the relative rate of starch loss is affected (1) by the length of previous storage at 53° F. and (2) by the ripening temperature, the hemicellulose relative loss rate is, apparently, independent both of the length of storage at 53° F. and of the ripening temperature.

It has previously been shown (Barnell, 1941*a*) that as a result of the decelerating effect produced by chilling, the starch content of the pulp and skin of chilled fruit is generally higher and that of total sugars generally lower than in unchilled fruit at stages during ripening comparable on the basis of appearance. The present data show that the hemicellulose content of the pulp of ripe chilled fruit is also lower than in good quality fruit.

TABLE VI

Composition (% of fresh weight) of the Skins of heavy ¾-full Fruit during Storage and Ripening at 53° F.

Days from cutting.	Total alcohol-insoluble residue.	Starch.	Cellulose (insoluble in N/75 HCl).	Pectin (as calcium pectate).	Hemicellulose.	Starch plus Hemicellulose.
1	10·91	2·68	2·66	0·69	4·88	7·56
2	10·52	2·67	2·72	0·46	4·67	7·34
9	10·18	2·04	2·94	0·62	4·58	6·62
14	9·89	2·70	2·81	0·64	3·74	6·44
17	9·89	1·60	3·08	0·56	4·65	6·25
21	10·66	2·07	3·37	0·64	4·68	6·65
25	9·87	1·62	3·41	0·50	4·34	5·96
29	9·74	1·72	3·77	0·58	3·67	5·39
33	9·17	1·24	4·11	0·63	3·19	4·43
37	7·16	1·07	3·17	0·42	2·50	5·57
44	9·16	0·41	4·62	0·57	3·56	3·97
51	10·95	0·58	5·60	0·79	3·98	4·56
58	11·70	0·56	5·85	0·90	4·39	4·95
75	13·63	0·71	6·64	0·69	5·79	6·50
85	13·92	0·84	7·46	0·54	5·08	5·92
93	13·21	0·62	6·82	0·37	5·40	5·02
99	15·00	0·50	7·66	0·38	6·36	6·96
Significant difference:	—	0·204	0·236	—	0·550	—
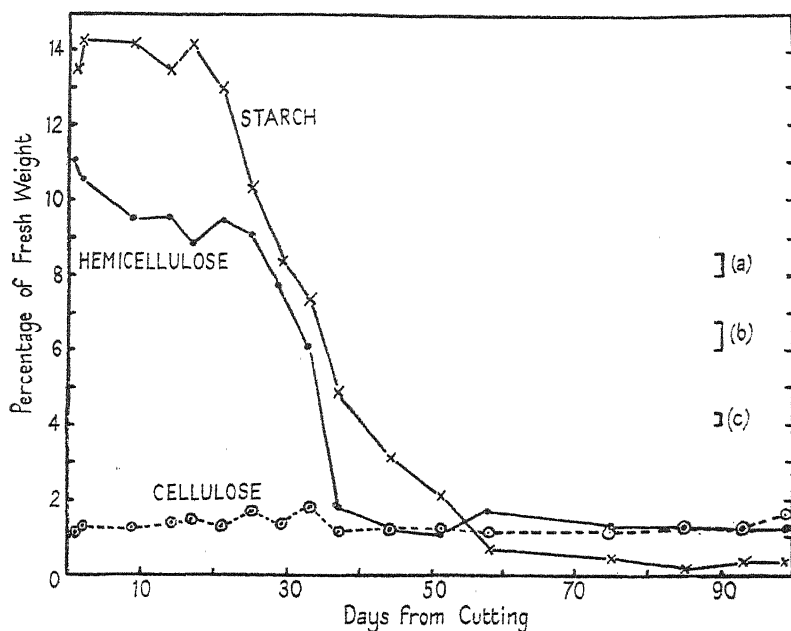
FIG. 6. Pulp. Starch, hemicellulose, and cellulose as percentages of the fresh weight during continuous storage of heavy ¾-full fruit at 53° F. The vertical lines (*a*), (*b*), (*c*) have the same significance as in Fig. 2.

The fruit remained firm and green till day 19, colouring, with chill colours, becoming increasingly evident from day 19 to day 33. From day 33 to day 59 the skin colour was bronze, from day 59 onwards the skin blackened and the fruit became increasingly soft.

The behaviour of 'cellulose', the final residual material after hydrolysis with dilute acid, was in contrast to that of hemicelluloses. During the storage periods at 53° F. the percentage of cellulose in the pulp in both grades of fruit showed little change; during the ripening periods at 68° F. for both
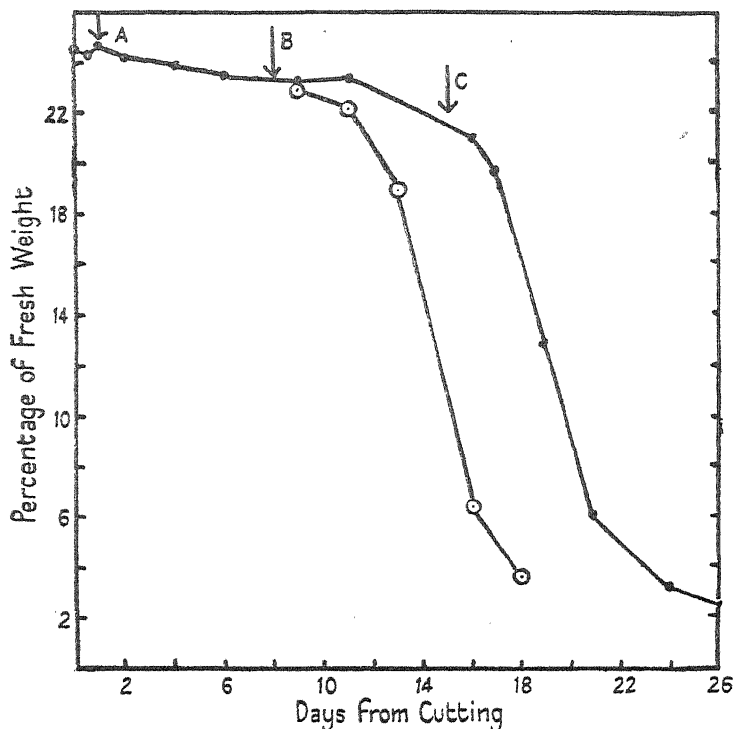
FIG. 7. Pulp. Starch *plus* hemicellulose as percentage of the fresh weight in the pulp of heavy ¾-full fruit during storage at 53° F. and ripening at 68° F. The arrows *A, B, C* have the same significance as in Fig. 2.

TABLE VII

Index of Starch Loss Rate (K_s) and Hemicellulose Loss Rate (K_h) during Storage at 53° F.

Grade.	Days.	K_s.	K_h.
Heavy ¾-full	99	0·0286	0·083
,,	14	0·135	0·083
,,	7	0·180	0·083
Standard ¾-full	20	0·130	0·083
,,	14	0·145	0·083

long- and short-stored fruit a small decrease was observed. There was no discernible difference in the cellulose content between ripe long-storage and short-storage fruit. The relevant data are found in Tables I and III. There was little change in the cellulose, as percentage of fresh weight, throughout the whole period for the fruit stored and ripening at 53° F.

Using the data in the form of total amounts per single pulp[1] to eliminate, so far as possible, the variation introduced by trends in water content, the loss of cellulose in the pulps during the ripening periods was calculated to be
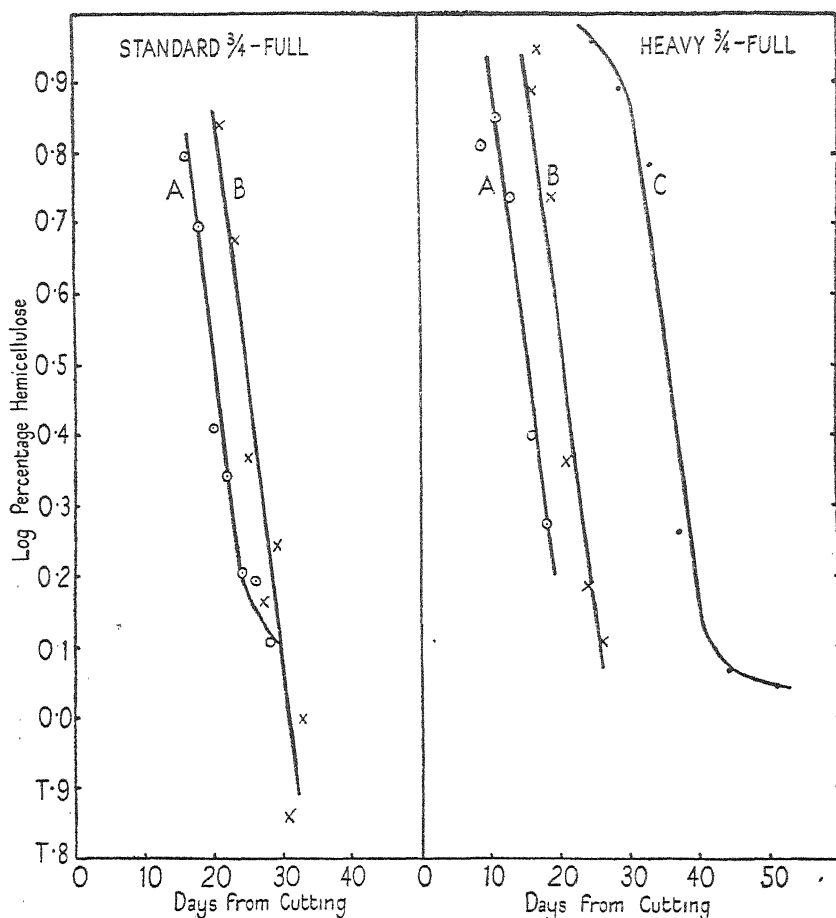
FIG. 8. Pulp. Logarithms of the hemicellulose as percentage of fresh weight plotted against time for the periods during which the percentages were decreasing rapidly. *A* and *B* are the curves for short- and for long-storage fruit ($\frac{3}{4}$-full and heavy $\frac{3}{4}$-full) ripening at 68° F. after storage at 53° F., *C* is the curve for heavy $\frac{3}{4}$-full fruit stored continuously at 53° F.

approximately 20 per cent. It would appear then that either (*a*) the cellulose as estimated is not homogeneous, a small part being more labile than the bulk, or (*b*) during the period of high hydrolytic activity which is characteristic of the banana pulp during ripening, the enzyme complex contains or develops a cytase which attacks the skeletal material of the organ causing its partial hydrolysis.

[1] Data for the bananas stored continuously at 53° F. given in Table IX, data for other treatments not given.

AMOUNTS OF HEMICELLULOSE AND CELLULOSE IN THE SKIN

Figs. 3, 5, and 10 show in graphic form the data of Tables II, IV, and VI for the amounts of hemicellulose and cellulose as percentages of the fresh
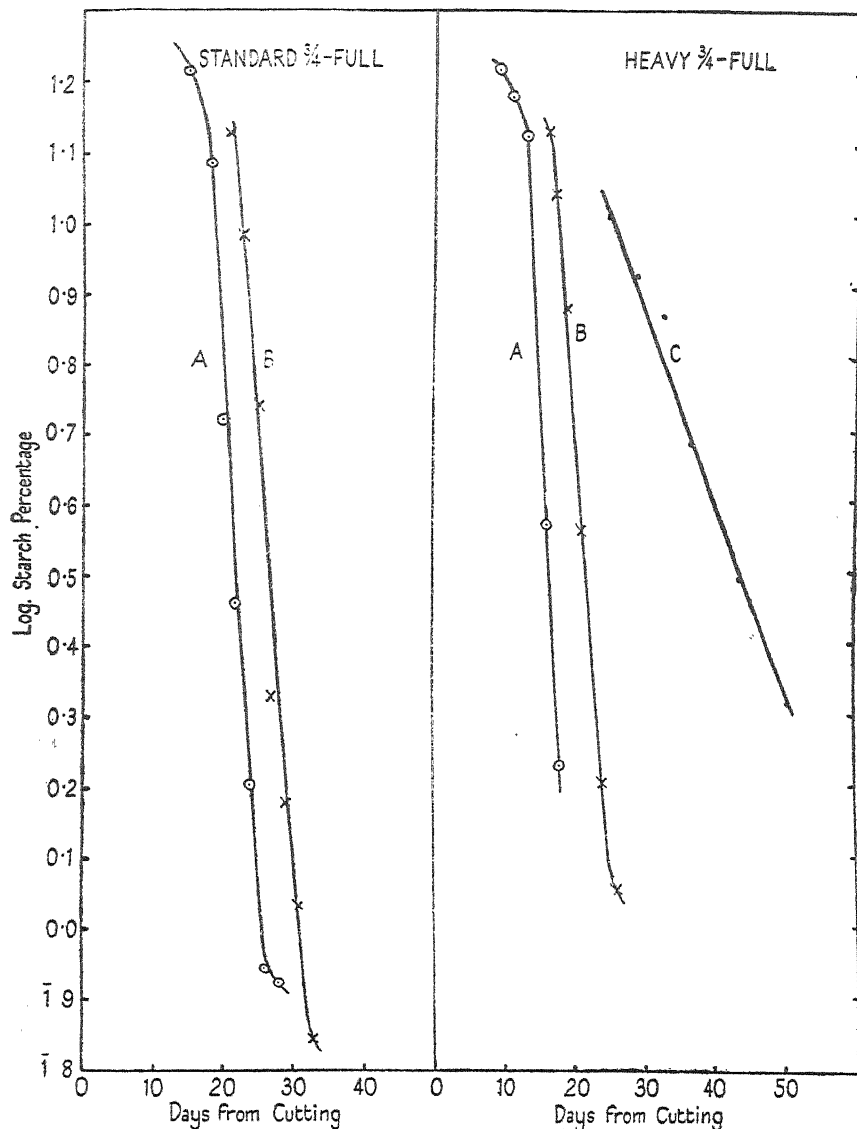
FIG. 9. Pulp. Logarithms of the starch percentages plotted against the times corresponding to those in Fig. 8. *A* and *B* are the curves for short- and for long-storage fruit respectively (¾-full and heavy ¼-full) ripening at 68° F. after storage at 53° F., *C* is the curve for heavy ¼-full bananas stored continuously at 53° F.

weight of the skin with the corresponding observations for the starch percentage for comparison.

The hemicellulose percentage in the skins of both grades of fruit under all five conditions (standard ¾-full long and short storage; heavy ¾-full long and short storage and storage continuously at 53° F.) showed relatively small changes compared with those in the pulp. An early slight increase in hemicellulose was observed during the period at 53° F., the percentage attaining
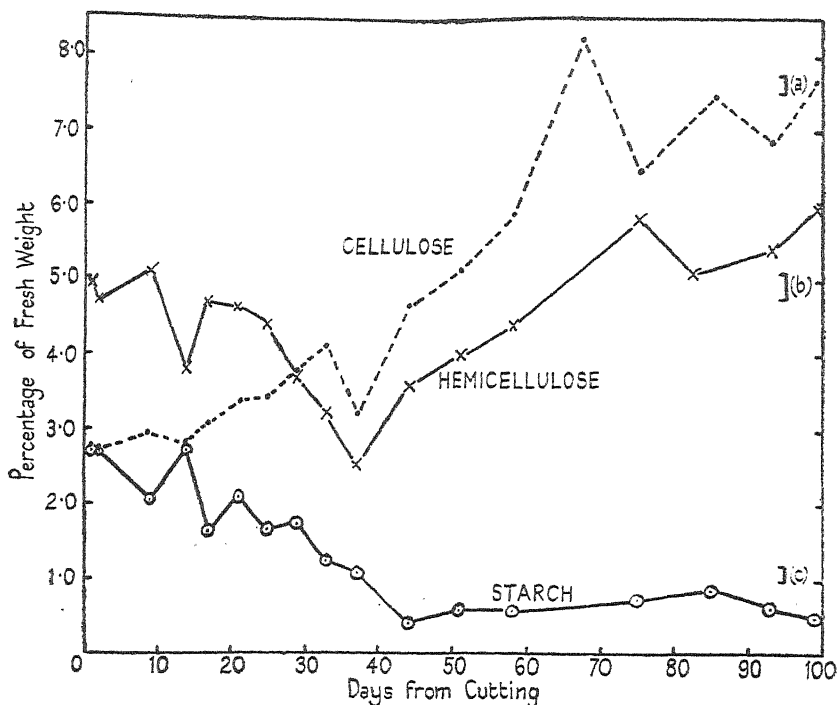
FIG. 10. Skin. Starch, hemicellulose, and cellulose as percentages of the fresh weight during continuous storage at 53° F. The vertical lines (a), (b), (c) have the same significance as in Fig. 2.

a maximum value on the 6th day in the heavy ¾-full fruit (Fig. 3) and on the 3rd day in the ¾-full fruit (Fig. 5). As in the pulp this early change of the hemicellulose percentage appeared to be complementary to the early changes in the starch. This is particularly clearly shown in Fig. 3 for the heavier grade of fruit, where the starch percentage fell steadily until the 6th day, and then rose; while hemicellulose rose to the 6th day and then fell. The sum of the percentage amounts of starch and hemicellulose showed a slow decrease over this period. The sampling intervals were not sufficiently close for these early changes in the hemicellulose to be observed in the fruit stored continuously at 53° F. (Fig. 10). The percentage of cellulose, shown in Figs. 3, 5, and 10, increased relatively slowly during the unripe stage.

The drifts of percentage amounts of hemicellulose and cellulose are to a considerable extent reflections of the underlying drift of the water content of the skin which loses a considerable quantity of water during ripening (Barnell,

Table VIII

Amounts (gm.) of Hemicellulose and Cellulose per single Skin during Storage at 53° F. and Ripening at 68° F.

	Heavy ¾-full fruit.					Standard ¼-full fruit.			
Days from harvest-ing.	Tempera-ture ° F.	Skin wt.	Hemi-cellu-lose.	Cellu-lose.	Days from harvest-ing.	Tempera-ture ° F.	Skin wt.	Hemi-cellu-lose.	Cellu-lose.
0	Room temp. (80–85°)	62·8	1·75	1·86	0	Room temp. (80–85°)	58·2	1·80	1·77
16 hr.	,,	62·3	1·92	1·78	20 hr.	,,	57·4	1·86	1·80
23 hr.	53°	62·0	2·17	1·84	26 hr.	53°	57·3	1·89	1·80
2	,,	60·2	2·02	1·74	3	,,	56·3	1·98	1·76
4	,,	60·5	2·18	2·03	6	,,	56·5	1·83	1·97
6	,,	60·2	2·40	1·89	10	,,	56·9	1·71	2·13
9	,,	59·0	2·03	1·76	14	,,	56·7	1·01	2·19
11	,,	59·0	2·09	1·94	17	,,	48·2	1·17	1·90
14	,,	48·0	1·60	1·69	19	,,	45·8	1·50	1·85
16	68°	46·7	1·60	1·65	21	68°	46·3	1·53	1·77
17	,,	56·1	1·83	2·16	23	,,	46·7	1·11	2·16
19	,,	52·4	1·35	2·16	25	,,	43·6	1·31	1·82
21	,,	43·2	1·40	1·88	27	,,	37·4	1·22	1·94
24	,,	38·8	1·19	1·90	29	,,	36·8	1·25	1·99
26	,,	35·3	1·23	2·04	31	,,	34·9	1·29	1·99
					33	,,	33·8	1·61	1·71
9	68°	58·3	1·77	1·83					
11	,,	58·8	1·59	2·01	16	,,	56·2	2·04	1·80
13	,,	56·2	1·69	1·88	18	,,	56·1	1·81	1·79
16	,,	49·7	1·16	1·81	20	,,	50·8	1·44	2·04
18	,,	45·9	1·16	1·82	22	,,	47·4	1·38	1·93
					24	,,	43·7	1·30	1·95
					26	,,	39·8	1·19	2·01
					28	,,	38·3	1·38	1·91

Table IX

Amounts (gm.) of Hemicellulose and Cellulose per single Pulp and single Skin of heavy ¾-full Fruit stored and ripened at 53° F.

Days from cutting.	Pulp wt.	Pulp Hemi-cellulose.	Cellu-lose.	Skin wt.	Skin Hemi-cellulose.	Cellu-lose.
1	97·1	10·71	1·15	61·6	3·01	1·64
2	96·6	10·10	1·28	61·7	2·89	1·67
9	96·4	9·21	1·21	60·8	3·09	1·79
14	94·7	9·05	1·28	60·1	2·24	1·69
17	93·3	8·30	1·38	59·9	2·79	1·84
21	93·6	8·89	1·22	60·3	2·76	2·03
25	95·2	8·68	1·62	52·3	2·30	1·78
29	93·4	7·27	1·26	54·4	2·00	2·05
33	92·7	5·64	1·71	50·1	1·60	2·06
37	95·6	1·75	1·10	46·6	1·16	1·47
44	94·2	1·09	1·16	40·9	1·46	1·89
51	95·8	1·06	1·24	38·2	1·52	2·14
58	100·9	1·75	1·16	36·9	1·62	2·16
75	94·1	1·25	1·06	32·5	1·88	2·09
85	88·7	1·16	1·10	30·7	1·56	2·29
93	79·1	0·97	1·02	32·8	1·77	2·24
99	80·4	1·08	1·33	33·4	2·16	2·56

1941, 1941*a*, 1942). When the data are presented in the form of total amounts per single skin (Tables VIII and IX) it is observed that for both grades of fruit there was little change in the amount of hemicellulose and cellulose during the unripe stage in storage at 53° F.

After the initial rise and subsequent falling phase the hemicellulose per-

TABLE X

Amounts (gm.) of Dry Matter, total Estimated Substances, total Estimated Carbohydrates plus Hemicellulose, and total estimated Carbohydrates per whole Finger of heavy ¾-full Fruit stored and ripened at 53° F.

Days from cutting.	Total dry matter.	Total estimated substances.	Total estimated carbohydrates *plus* hemicellulose.	Total estimated carbohydrates.
1	34·30	32·60	28·60	14·88
2	34·34	32·31	28·58	15·59
9	33·79	32·25	28·07	15·77
14	33·13	31·22	27·09	15·80
17	33·53	31·26	26·99	15·90
21	33·72	31·54	27·24	15·59
25	31·72	29·45	25·08	14·10
29	31·58	27·88	23·42	14·15
33	28·78	25·04	20·59	13·35
37	25·09	21·80	18·50	15·59
44	24·73	21·15	17·28	14·72
51	24·37	19·98	15·75	13·17
58	25·35	21·20	16·91	13·54
75	23·23	19·62	15·67	12·54
85	21·53	17·77	13·77	11·05
93	19·12	15·01	11·26	8·52
99	20·92	17·67	13·29	10·05

centage in the skin tended to rise during the eating-ripe and over-ripe stages. In the fruit stored continuously at 53° F. (Fig. 10) the falling stage was from approximately the 17th to the 37th day and the rate of fall of the hemicellulose percentage was clearly greater than that of starch (as also occurred in the pulp). The percentage of cellulose increased more rapidly during the ripening period than during the unripe stage.

Using the data in the form of total amounts per skin (Tables VIII and IX) it is observed that during ripening at 68° F. or at 53° F. there was a loss of hemicellulose ranging from 20 to 50 per cent. according to grade and treatment. The data for the fruit stored continuously at 53° F. are presented graphically in Fig. 11. Compared with the pulps of ripening bananas these changes in the amounts of hemicellulose in the skins are relatively small. They are also small relative to the changes in the amounts of starch in the skin (Barnell, 1941*a*, 1942). The total amounts of cellulose in the skin showed some fluctuations but no distinct trends during ripening; this is true for both grades of fruit receiving long- or short-storage treatments and for the heavy ¾-full fruit stored continuously at 53° F. (Fig. 11).

The skin of the banana, unlike the pulp, appears to maintain its skeletal

material (cellulose) unaltered throughout ripening and even through the over-ripe stages. Either the cellulose of the skin is more homogeneous than that of
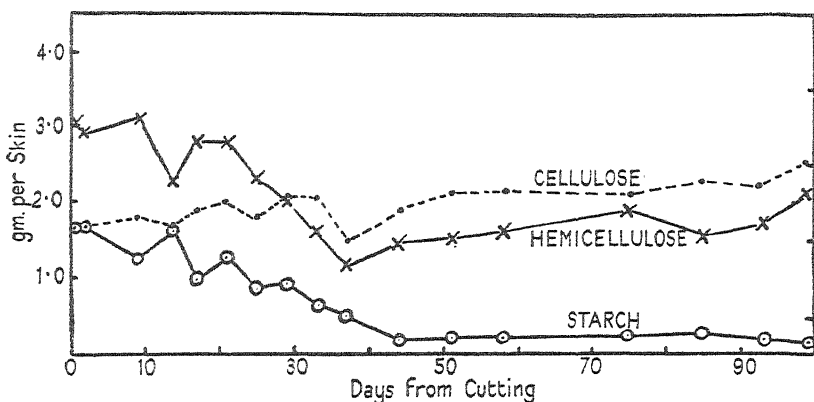
FIG. 11. Skin. Amounts (gm.) of starch, hemicellulose, and cellulose in skins of single fingers during continuous storage at 53° F.

the pulp or, alternatively, no cytase is developed in the complex of hydrolytic enzymes in the skin.

Losses of Total Dry Matter and Estimated Substances in Whole Fingers at 53° F.

In previous work (Barnell, 1942) it was observed (i) that during continuous storage at 53° F. the rate of loss of total dry matter considerably exceeded that estimated as liberated carbon dioxide of respiration; it was also observed (Barnell, 1942, Fig. 8) (ii) that up to 17 days after cutting there was little change in the amount per single finger of total estimated carbohydrates (starch+sucrose+fructose+glycosidic-glucose), but between 17 and 25 days the loss of these exceeded the loss of dry matter; (iii) this stage was followed by a phase (30 to 40 days) of *increasing* amounts of total estimated carbohydrates during which the total dry matter fell at an increased rate. Subsequently both total dry matter and total estimated carbohydrates fell at approximately the same rates.

The present work has to some extent clarified the problems raised by the above observations. The accumulated data on the carbohydrates of bananas at 53° F. have been collated in Table X (derived from Barnell, 1942, Tables V and VI and Table IX of the present contribution). Column 2 consists of the sums of the total dry matter for pulp and skin giving values for whole fingers, column 3 consists of the sums of all the estimated substances (starch, sucrose, glucose, fructose, glycosidic-glucose, hemicellulose, cellulose, pectin as calcium pectate, acid as malic acid), column 4 contains the values for the sums of total estimated carbohydrates (starch, sucrose, glucose, fructose, glycosi-dic-glucose) plus hemicellulose, while column 5 consists of values for the total

estimated carbohydrates. The values in these four columns have been plotted in Fig. 12 as the four curves *A*, *B*, *C*, and *D*, respectively (*A* and *D* appeared in Barnell, 1942, Fig. 8).

A comparison of curves *A* and *B* shows that during the first 25 days approximately 94 per cent. of the amount of total dry matter is accounted for
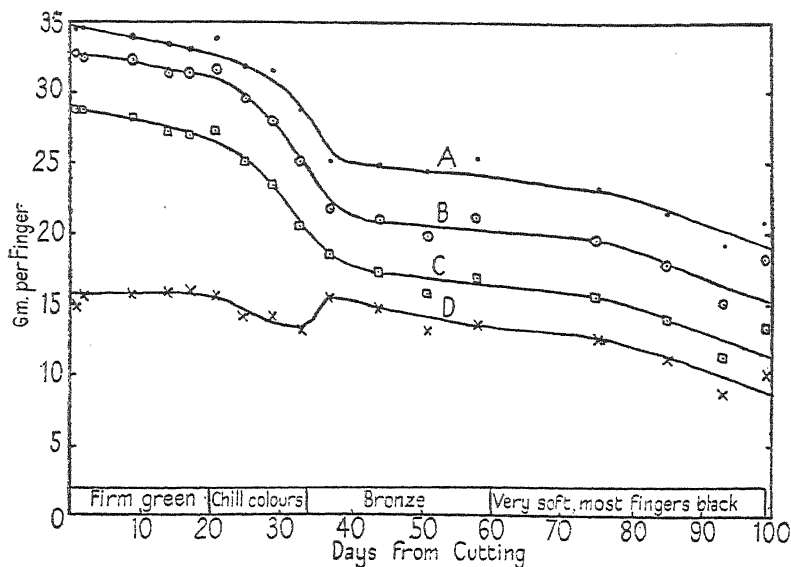
FIG. 12. Whole finger. Stored continuously at 53° F.: *A*, dry matter in pulp and skin; *B*, the sum of all estimated substances, namely, starch, sucrose, glucose, fructose, glycosidic-glucose, hemicellulose, cellulose, pectin as calcium pectate, acid as malic acid; *C*, the sum of total estimated carbohydrates (starch, sucrose, glucose, fructose, glycosidic-glucose) plus hemicellulose; *D*, total estimated carbohydrates (starch+sucrose+glucose+fructose+glycosidic-glucose).

by the total estimated substances; after 25 days there is a divergence of the curves and the proportion falls to approximately 84 per cent. It would appear that during the major changes in metabolism there is a transformation of some estimated substance or substances to one or more substances not subsequently estimated. The parallelism of curves *B* and *C* indicates that cellulose, pectin, and acid show relatively slight quantitative changes throughout the whole period at 53° F. on the basis of amounts per whole finger. It is seen from curves *C* and *D* that the hemicellulose fraction (*C*−*D*) is the only one which undergoes changes similar in kind and magnitude to the total estimated substances (*B*). Between 20 and 33 days there was a relatively rapid loss of total estimated carbohydrates (*D*), but this was immediately followed by an increase in these substances due to a rise in the total sugars, presumably originating from the hemicellulose.

The amount of the hemicellulose fraction (*C*−*D*) disappearing throughout storage at 53° F. is more than adequate to account for the loss observed in

the total dry matter (*A*), but the fate of the disappearing hemicellulose remains unknown. A portion may reappear in some other form while the remainder is lost from the finger. The amount of dry matter lost has already been shown (Barnell, 1942) to be greatly in excess of that required for carbohydrate loss calculated from the observed rate of carbon dioxide liberation. It should be noted that certain differences in treatment and storage conditions were present in the bananas used for carbohydrate estimation and for respiration determinations: (1) the fingers were attached to the bunch until the moment of sampling, and the possibility of transference of sugars and other soluble substances to the main stalk must be considered a possible explanation; (2) the bunches were at a lower humidity (approximately 80 per cent.) than the fingers used for the respiration experiments (saturated atmosphere); (3) there was a delay of approximately 24 hours before the fruit for carbohydrate estimations was placed in cold storage.

Alternative possibilities to account for the discrepancy are: (*a*) that relatively large quantities of volatiles are formed and liberated during the period of colouring of the skin; (*b*) that as suggested previously (Barnell, 1942), the method of determining total extracted residue was such that any relatively volatile substances found in the tissues would be lost and hence differences between dry-matter weights would include these losses; (*c*) any appreciable proportion of anaerobic respiration would render actual dry-matter loss greater than that calculated from carbon dioxide liberation data on the basis of completely aerobic respiration.

Samples from a large population of fingers detached from the bunch at the start of the experiment could be used to determine whether transference of materials from finger to main stalk occurred, while the drying of tissues at low temperatures *in vacuo* to reduce the possible loss of volatile substances could be used to reduce this loss in the present method.

It is hoped that it may be possible to carry out the necessary experiments and also that respiration data in which values for the respiration quotient are included, may be obtained.

DISCUSSION

With increasing knowledge of the metabolism of fruit in storage, substances whose physiological significance was hitherto little considered or whose presence was unsuspected are increasingly found to be important. The interest in investigating these substances lies both in the elucidation of their part in metabolic processes and in the more utilitarian aspect of their role in composition and quality. This applies to substances present in small amount such as the nitrogenous constituents of the apple (Hulme and Smith, 1938) in which fruit, though it is present in very small amount, the 'protein' fraction is closely related to the respiratory activity both of the apple as a whole and of the individual cell; this fraction is therefore important in connexion with the storage properties of the apple. Sorbitol is present in

relatively considerable quantity in pears (Kidd, West, Griffiths and Potter, 1940) and plums (Donen, 1939; Donen and Roux, 1939). It decreases in Conference pears during storage and the disappearance is accompanied by an almost equivalent increase in fructose. In the plum changes in the sum of sorbitol, acid, and starch account, over long periods, for the loss of carbon by respiration. Tannins are present in the banana in small quantity, but appear to be important both in metabolism and quality.

Pulp. In the pulp of the banana hemicelluloses have been shown to be present in very considerable quantity, about 8 to 10 per cent. in the green fruit, and to decrease quickly to a relatively small percentage (1 to 2 per cent.) during the ripening period. This period, which immediately follows the attainment of the sprung condition by the fruit, is one of intense metabolic activity. Respiration rate is high, the pulp is receiving water from the skin, and hydrolytic activity is great. Starch quickly decreases in amount during this stage whilst sugars and acid increase (Barnell, 1941, 1941a). The disappearance of hemicelluloses at this time is due, presumably, to hydrolysis, and so part of the sugar and acid accumulation may be ascribed to them. Hemicelluloses in the banana pulp must be considered to be reserve carbohydrates quantitatively of importance comparable with starch, giving rise on hydrolysis to substances which may serve as substrates for the respiratory process. They thus appear to be labile reserve carbohydrates similar to those described by Buston (1935). During recent years it has become increasingly clear that hemicelluloses are present not necessarily solely or throughout the life-history as constituents of the skeletal material of plant tissue, though some may be, but can serve as sources of energy in the plant's metabolism. Thus, for example, Norman (1937): 'It seems probable that the hemicelluloses provide a more easily available source of energy than does cellulose.' Also, investigations into the changes taking place during the aerobic rotting of plant materials have shown that hemicelluloses suffer an early rapid loss and are extensively removed in any completed decomposition (Tenny and Waksman, 1929; Norman, 1929).

At the time the percentage amount of hemicelluloses in the banana pulp decreases the titratable acid (Gane, 1936; Barnell, unpublished) rises and the pH of extracts falls. Hemicelluloses on hydrolysis may give rise to any or all of the following types of substances: hexoses, pentoses, and uronic acids. If it be postulated that uronic acids on partial oxidation, such as might occur at a time when the oxidation potential is low, give rise to dibasic acids, e.g. malic or succinic, then it appears highly probable that the increasing acid content of the banana pulp during the ripening of the fruit is derived in part from the katabolism of hemicelluloses (cf. Donen, 1936).

The behaviour of hemicelluloses in the pulp differs from the behaviour of starch in an important respect. It has been observed that the starch relative loss rate in the pulp of a banana is affected by the duration of storage at 53° F. and by the temperature at which ripening takes place. By contrast the hemicellulose relative loss rate in the pulp at 68° F. is (within the limits of

accuracy of estimation by the present methods) independent of the duration of previous storage at 53° F. and even remains the same when ripening occurs at 53 ° F. The initiation of rapid hemicellulose hydrolysis in the pulp is delayed by storage at 53° F. to approximately the same extent as is that of rapid starch hydrolysis, but, once this rapid hydrolysis has begun, the hemicellulose disappears at the same rate whatever the previous history of the fruit, whereas prolonged storage at 53° F. decreases the rate of starch hydrolysis either at that temperature or subsequently at 68° F. The relatively *sudden* change in a banana finger from the turgid green condition to the sprung condition, whatever the temperature of storage (within the limits so far investigated), is probably a result of this relatively unchangeable hemicellulose hydrolysis rate. A secondary effect is the relationship between the different constituents in ripe fruit after various storage treatments. One result of chilling is a higher starch : hemicellulose ratio since the rate of disappearance of starch is affected by the storage treatment while the hemicellulose falls to the eating-ripe percentage at a rate irrespective of any chilling sustained. The hemicellulose percentage appears to be of significance as a quality factor chiefly in affecting the texture of the pulp. Bananas stored continuously at 53° F. eventually 'ripen' to a condition where the starch and sugar percentages correspond fairly closely with those recognized as characteristic of eating-ripe fruit, but the hemicellulose percentage is then extremely low, the pulp is of a watery consistency and the flavour still astringent, and the skin is black with fungus mycelium appearing.

Skin. In the skins small changes occurred in the hemicellulose percentage during the first few days of the period at 53° F. and these, as in the pulp, were complementary to those of starch. Most of the changes observed in the percentage amounts, particularly during the ripening phases, were reflections of changes in the water content. The change in absolute amount of hemicellulose in the skin during ripening was a decrease approximately to half the original amount present—a relatively small change compared with that in the pulp. Although, as in the pulp, the titratable acid increased to some extent, the pH values of the aqueous extracts tended to rise simultaneously (Barnell, unpublished) but with a fairly wide scatter, suggesting considerable buffering powers within the skin tissue and the absence of increasing amounts of free acid.

No appreciable changes were detected in the absolute amounts of cellulose in the skin during the various storage and ripening treatments. Also no appreciable differences were noted in the absolute amounts of either hemicelluloses or cellulose between long- and short-stored fruit, though the water-content changes were sufficient to produce differences in the percentage data.

During the phase of active hydrolysis of starch and hemicellulose in the pulp a certain amount of cellulose (approximately 20 per cent.) disappeared. In the skin no such loss of this skeletal material was observed. It would appear that either (1) the complex of hydrolytic enzymes which became active in the pulp at this time contained cytase while that in the skin did not,

or that (2) the cellulose, as estimated, was, in part, different in the skin from that in the pulp, the latter containing a relatively small fraction more labile than the major portion common to both pulp and skin.

Whole Finger. The considerable loss in total dry matter during ripening previously recorded in bananas continuously stored at 53° F. (Barnell, 1942) has now been shown to be due to a loss of hemicellulose, the decrease in which in the whole finger, without equivalent increase in sugars, is more than adequate to account for the amount of dry matter disappearing. However, the fate of this disappearing hemicellulose remains unsolved. A part reappears as dry matter in other forms, but the remainder is lost by the finger. The loss of carbohydrate as carbon dioxide of respiration has been calculated from discrete respiration measurements (Barnell, 1942) and found to be much too small to account for more than a small part of the dry matter lost.

Alternative hypotheses to account for the excess dry matter loss over respiration requirements are: (1) transference of hydrolysis products from the finger to the main stalk; (2) formation and loss of relatively large quantities of volatiles during the ripening phase; (3) the method of determination of the dry matter: this involved drying and weighing the alcohol-extracted residue and drying and weighing aliquots of alcohol extract, both being dried at 100° C.; (4) the occurrence of anaerobic respiration as a considerable proportion of the total respiration with accumulation of alcohol and possibly other substances.

Present practice in the transport of bananas consists essentially of prolonging the pre-climacteric phase; post-climacteric fruit cannot be easily handled owing to bruising and dropping of the fingers. Future investigations must aim at the further prolongation of the pre-climacteric phase and also attempt to protract the climacteric itself. They will necessitate attempts (i) to delay the onset of rapid starch and hemicellulose hydrolysis, (ii) to slow down the rate of hemicellulose hydrolysis during the climacteric to preserve a balance with that of starch, and (iii) to reduce the development of astringency in the ripening of fruit after prolonged refrigerated storage. The possible lines of such investigations have previously received mention (Barnell, 1942) and will include low-temperature storage in controlled atmospheres. It is possible that low oxygen partial presssures will reduce the rate of hemicellulose hydrolysis and also decrease the amount of astringent tannins; in some fruits, e.g. persimmons, high carbon-dioxide containing atmospheres are employed to render the tannins non-astringent; high carbon-dioxide partial pressures may be found, on investigation, to decrease hemicellulose hydrolysis rate in the banana. Low oxygen partial pressures with or without high carbon-dioxide partial pressures may also reduce oxidation of polyphenolic substances in the skin, thus decreasing browning and blackening.

SUMMARY

1. The changes in the amounts of hemicellulose and cellulose in the pulps and skins of two commercial grades of Gros Michel bananas have

been followed during short or long storage at 53° F. followed by ripening at 68° F. and also, for one grade, during continuous storage at 53° F.

2. The pulp of the green banana contains 8–10 per cent. fresh weight of hemicellulose which declines rapidly to 1–2 per cent. during ripening.

3. The rates of hydrolysis of hemicellulose and starch at 53° F. are slow prior to the onset of the changes which constitute ripening.

4. The incidence of the change from slow to rapid hydrolysis of hemicellulose at 53° F. or at 68° F. coincided with that of the increased rate of starch hydrolysis which, in turn, has previously been shown to synchronize with the increased rate of sugar formation.

5. Once the changes accompanying ripening have been initiated the new increased rate of hydrolysis of hemicellulose is apparently unaffected by temperature, identical relative rates being observed at 68° F. and at 53° F. This contrasts with the relative rate of hydrolysis of starch, which furthermore is affected by the duration of the previous storage at 53° F.

6. This differential effect of temperature upon the relative rates of disappearance of starch and hemicellulose results in a high starch : hemicellulose ratio in the pulps of chilled bananas. Future investigations designed to extend the storage life of the banana should therefore include attempts to reduce the hemicellulose hydrolysis rate to preserve the balance with that of starch. Possible lines for such investigations are discussed.

7. The pulp of the green banana contains 1–3 per cent. fresh weight of cellulose, this changes little during storage at 53° F. During ripening at 68° F. a slight decrease occurred. There was no apparent difference between fruit stored for long or short periods at 53° F. Expressed as total amounts per single pulp approximately 20 per cent. of the cellulose disappeared at 68° F. following the two storage treatments.

8. The skin contained much less hemicellulose than the pulp and the decrease in absolute amount of hemicellulose during ripening was relatively small. No appreciable differences were noted in the absolute amounts of hemicellulose between long- and short-stored fruit, though the decrease in water content was sufficient to produce differences in the percentage data on the basis of fresh weight.

9. Cellulose showed no appreciable change in absolute amount in the skin either at 53° F. or at 68° F., but decreases in water content were sufficient to produce increases in percentage amounts and differences in percentage amounts between long- and short-stored bananas when ripe.

10. The amount of hemicellulose disappearing without equivalent increase in sugars in the banana finger during ripening is more than adequate to account for the loss of total dry matter during this period. It is suggested that some of the hydrolysis products of hemicellulose serve as substrates for respiration while others, on oxidation, give rise to dibasic acids.

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The Fossil Conifer Elatides williamsoni

BY

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With Plate VIII, and three Figures in the Text

INTRODUCTION

ALTHOUGH *Elatides* (*Pagiophyllum*) *williamsoni* has long been known and is a common fossil in the Gristhorpe Plant Bed, the material has never been closely investigated and it has thus remained unclassifiable. The present account which is based largely on the Edwards and Wonnacott collections in the Department of Geology of the British Museum shows that it is a typical member of the Taxodiaceae, the oldest yet found. Its age is Middle Estuarine (Bajocian, Lower Oolites), and the locality of the present material is the Gristhorpe Bed, Cayton Bay, Yorkshire.

Nomenclature

The correct name of the shoots and cones described here is uncertain, but fortunately this involves no serious confusion. *Elatides* was founded by Heer (1876, p. 77) for female cones and shoots which resemble the present material and may prove to be essentially similar. No other species is known in Yorkshire which could be confused with *E. williamsoni*, but there are some species of sterile shoots included in the genus Pagiophyllum from other lands which are not yet satisfactorily distinguished.

Chief References

1828 *Lycopodites Williamsonis* Brongniart, p. 83 (name only).
1829 *Lycopodites uncifolius* Phillips, p. 147, pl. VIII, fig. 3 (shoot).
1833 *Lycopodites Williamsonis* Brongn., Lindley and Hutton, pl. XCIII (shoot, female cone).
1875 *Walchia Williamsonis* (Brongn.), Phillips, p. 230, pl. VIII, figs. 1, 3; lign 61 (shoot, male cone).
1884 *Pachyphyllum? Williamsoni* (Brongn.), Saporta, p. 306, pl. CLXII, figs. 1, 2 (shoot, female cone).
1897 *Elatides Williamsonis* (Brongn.), Nathorst, p. 34 (name).
1900 *Pagiophyllum Williamsoni* (Brongn.), Seward, p. 291, pl. X, figs. 2, 3, text-fig. 52 (shoots, female cone, male cone).
1919 *Elatides Williamsonis* (Brongn.), Seward, p. 271 (discussion).
For full literature to 1900 see Seward, 1900, p. 291.

Vegetative organs

Seward (1900) has figured and described excellent leafy shoots; their appearance is intermediate between *Sequoia gigantea* and *Cryptomeria japonica*. The leaves persist, even on twigs as thick as 6 mm., and are borne in a spiral
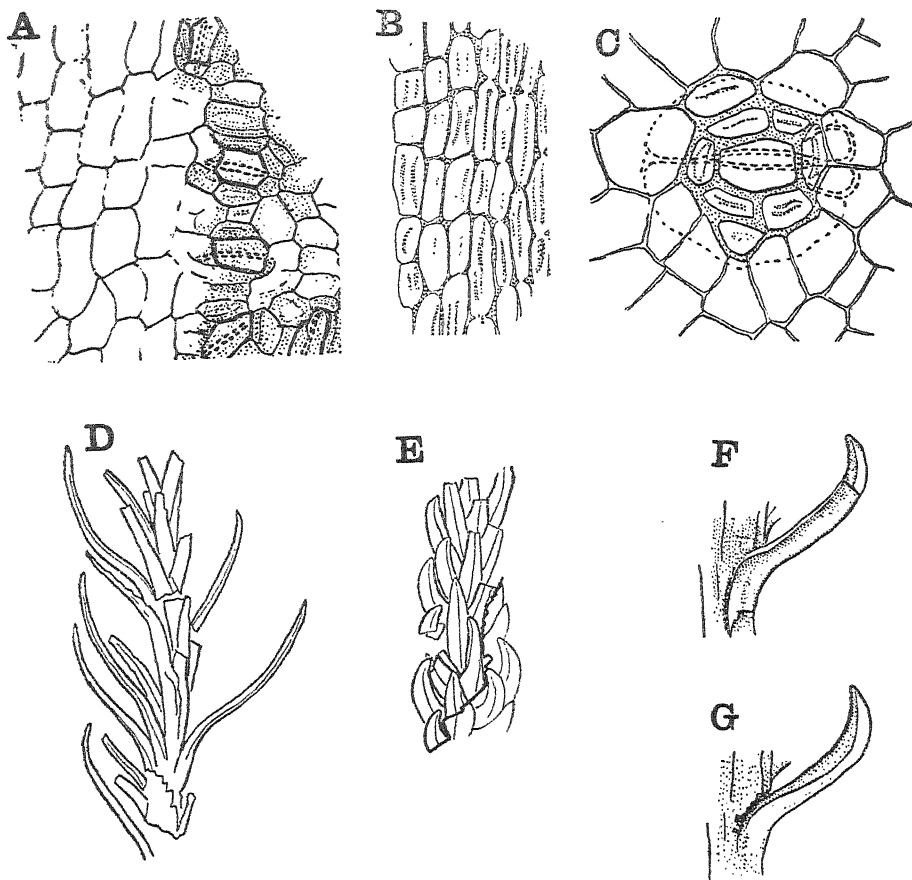
Text-Fig. 1. *Elatides williamsoni*, vegetative structure. A, lower cuticle of leaf showing part of a stomatal band. V 25876 (× 200). B, upper cuticle of leaf. V 25876 (× 200). C, stoma from scale of female cone. V 26852 (× 400). D, shoot with long leaves. V 25876 (× 2). E, shoot with short leaves. V 21885 (× 2).   F, leaf of medium length, the substance has fallen off the rock near the tip. V 25876 (× 4). G, the same as F, but the whole of the leaf substance has been removed to show the course of the lateral angles.

which may be 3/8, and apparently projected evenly round the twig instead of being flattened. Early descriptions of the leaves as 'opposite' and with 'stipular' appendages refer to specimens in which leaves along the front of the shoot have been broken by rock cleavage near their bases. The leaves arise from decurrent basal cushions which are, however, somewhat concealed by the free parts and have no petiolar constriction; the free part points at first out-

wards and then nearer the tip bends parallel with the stem. Their size and appearance vary (extreme forms are shown in Text-fig. 1, D, E), but they are always stiff and angular, a feature brought out strongly in Lindley and Hutton's figures. Comparison of the two sides of leaves compressed in different ways indicates that they were square in section and orientated so that corners point up and down. The vascular strand is not visible in the thick bituminous substance, but the epidermal cells are sometimes seen clearly. The stomata form well-marked bands on the two lower faces and these bands pass down on to the basal cushion, which is therefore like that of Sequoia where it is green and photosynthetic. In none of the leaves examined were any stomata recognized on the upper sides—an unusual feature in a conifer with thick leaves.

The cuticle has not been hitherto described. It is thin and rather ill preserved in the present material so that the following description is incomplete and based on fragments. The upper sides (Text-fig. 1, B) show more or less elongated straight-walled cells whose surface is commonly marked with a pair of longitudinal thick bars which may represent the sides of a compressed bulge. The under sides show similar cells, but their longitudinal bars are indistinct. The stomatal bands occupy about half the width of a lower side; here the epidermal cells are shorter, the surface more thickly cutinized, and the lateral walls project inwards (Text-fig. 1, A). The stomata are orientated irregularly, many being transverse, and are scattered, not forming longitudinal rows. The subsidiary cells form an irregular ring; some of them may be accompanied by an encircling cell, and their surface often shows a thin strip running parallel with the margin of the stomatal pit. The stomatal pit is wide and probably shallow; the cuticle of the guard cells seems remarkably extensive, but this is most likely due to some peculiar fat impregnation during preservation as some of the mesophyll may be preserved likewise.

The structure of the wood is unknown; we have at present no technique which can be used on this sort of material.

The comparative structure of the vegetative organs is discussed on p. 334.

Male cone

Phillips (1875) figured a detached male cone, and so in more detail did Seward (1900), who refers also to attached specimens being known. Detached specimens are extraordinarily common; there are over a hundred in this collection, but only a few specimens of rather young ones are borne on their parent shoots. They are borne in a terminal cluster of 3–6 on an ordinary twig, and their development was traced from a small oval bud (Pl. VIII, Fig. 5) through intermediates (Pl. VIII, Figs. 6, 7) to maturity (Pl. VIII, Fig. 1), but every one of the mature cones is detached. The mature male cone is about 23 mm. (15–28 mm.) long and about 5 mm. wide. It has no distinct stalk, and the cone axis seems fairly robust. The surface of the cone is covered by the imbricating heads of the sporophylls, and the surface of the axis shows ridges made by the decurrent bases of the sporophylls. The phyllotaxis of the

sporophylls appears to be 5/13, but in other specimens, perhaps as a result of twisting, they seem to arise in alternating whorls of about eight.
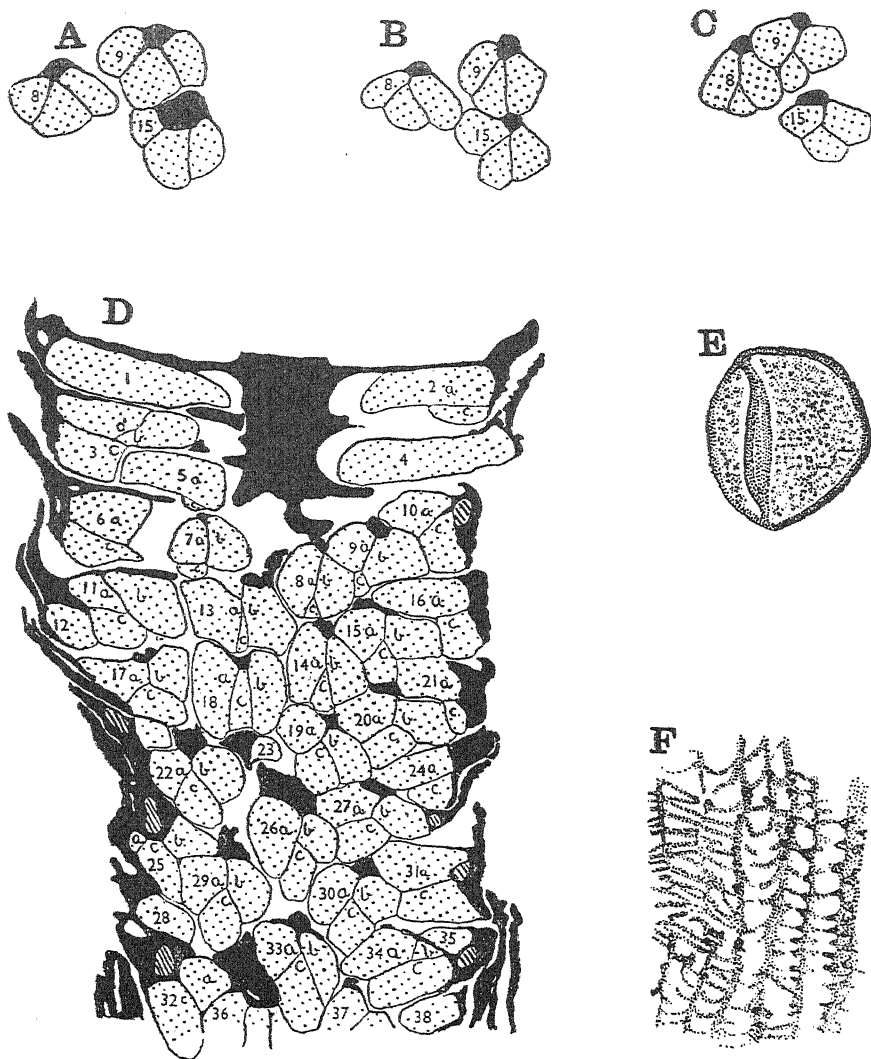
TEXT-FIG. 2. *Elatides williamsoni*, male cone. A, B, C, sections of three of the sporophylls shown in D, but at different levels. A, 180 μ above D near distal end; B, 120 μ above D; C, 160 μ below D, near proximal end. D, oblique-longitudinal section through nearly ripe male cone. Carbonaceous matter solid black; pollen sacs stippled, resin bodies with oblique lines. The sporophylls are numbered arbitrarily, and the three sporangia of each are labelled '*a*' (the left), '*b*' (the median), and '*c*' the right, all from V 26844 ($\times$ 40). E, pollen grain V 20401 ($\times$ 800). F, wall of pollen sac, from celloidin pull V 21375 ($\times$ 200).

The sporophyll has a rather stiff stalk terminating in an expanded, somewhat diamond-shaped head which is attached just above the bottom corner (Pl. VIII, Fig. 9). After the pollen has been shed the pollen sacs may

disappear and the stalk appears remarkably slender. The head has a thickened keel extending longitudinally to just below the apex (Pl. VIII, Figs. 4, 9). The bottom corner, is, however often concealed (Pl. VIII, Figs. 3, 8). The epidermal cells point towards these margins which are thin, probably one cell thick. The head has a cuticle showing the rectangular epidermal cells and on maceration yields a few rather large oval masses of resin about 200 μ long.

Sporangia

The sporangia are elongated sacs extending from the sporophyll head to the axis. Their structure is uncertain in mature cones because the walls collapse, but in certain young ones of about half the mature length their form and number was established by sectioning.[1] Each sporophyll bears three pollen sacs, two upper ones attached longitudinally to the stalk and one below which is joined to these two and is in contact with the head but not directly attached to the stalk. Evidence of continuity between the sporangial walls is provided by the rock matrix in the cone which is present in thin layers between the sporangia of different sporophylls but never between adjacent ones of the same sporophyll (Text-fig. 2, A–D).

In the ripe cone the pollen sacs have dehisced longitudinally and are empty. The wall then shows its single layer of cells clearly, and when detached by a celloidin pull shows cells with well-marked bands of thickening crossing their surface and extending up the sides (Text-fig. 2, F).

Pollen

Most of the pollen studied was obtained from half-sized immature cones, but in a few mature ones a single pollen sac had failed to open and yielded pollen. The grains are round, 30 μ in diameter, and show a longitudinal furrow. The extine is moderately thick, and smooth or very faintly dotted.

Female cone

Typical female cones have been figured by Seward. They are borne terminally on long or short shoots with normal foliage. Their mature size varies only slightly, one of the smallest, and also the widest seen are figured (Pl. VIII, Figs. 10 and 15), the longest (not figured) is 6 cm. long.

The cone scales form a phyllotaxis spiral, possibly 8/21. Most of the surface was covered by the tapering apices of the scales (Pl. VIII, Fig. 10), but as a rule the plane of rock cleavage removes the points, when the cone with

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[1] The sections shown in Pl. VIII, Fig. 2 and Text-fig. 2 were prepared as follows. A rock fragment showing the cone on its surface was impregnated with synthetic resin (R. 0014, kindly supplied by Bakelite Ltd.). The impregnation was done in a vacuum, but this may not be necessary. The resin was then hardened by baking for some days at about 50° C. and finally at 100° C. for a few hours. The surface of the rock and fossil was then gently polished away, using flour, emery, and paraffin on a glass slab. After cleaning, the face of the specimen was smeared with oil and observed under strong oblique illumination. Most of the tissues of the cone are black, the pollen sac walls dark and pollen grains light brown, the resin bright yellow, the rock matrix pale grey, and bakelite filling red.

truncated scales looks a good deal like a small Picea cone (Pl. VIII, Fig. 15). The cone scales are persistent and the stout cone axis is only seen after they have been dissected away (Pl. VIII, Fig. 18). The cone must have been fairly closely constructed, for but little rock matrix lies between successive scales.

Each scale consists of the following parts:

1. A basal part nearly 1 cm. long making an angle of 45° to the cone axis. Its substance is thick and it grows broader outwards. The surface shows some ridges on both sides but no definite organs till the seeds are reached on the upper side (Text-fig. 3, J, K). Large resin bodies occur in the substance.

2. The outer part about 7 mm. long making an angle of 15° with the cone axis. It widens to about 5 mm. just above its base and then contracts into a finely tapering apex. At its widest point the margins are exceedingly delicate. The tapering apex is of moderately thick substance, but thinner than the leaf, and may lie parallel with the cone axis. This part of the scale is smooth and is very easily dissected from the rock.

The seeds and the small 'ligular' outgrowths are borne on the upper side of the cone scale at about the junction of the two regions. They were best seen by dissecting away the scale from its back; attempts were made to section female cones, but with less success than with the male cones. In this region the seed placentae and the projecting ligules fix the substance to the matrix particularly firmly.[1]

The organs regarded as seed placentae form an arched row of conspicuous projections (Text-fig. 3, K). There are often five on a full-sized cone-scale, but probably fewer on the narrow scales near the apex of the cone. Only a few seeds were found in the ripe cones, and conclusive proof is lacking that they were borne on the placentae and that there were originally five. The micropyles of the seeds face the cone axis and the seeds are so wide that their margins must overlap considerably.

The 'ligular outgrowths' are delicate flaps of tissue attached a little above the placentae. In favourable dissections it was established that there are at least three of these flaps (Pl. VIII, Fig. 12) and it is likely that there may have been five, one above each seed. The substance of the flap is delicate at first and becomes a mere film, apparently one cell thick, near the apex.

The cone scales have fairly well developed cuticles, though only rather small fragments could be studied. The outer parts of the cone scales have numerous stomata rather like those of the leaf, but the stomatal bands are much broader, the guard cells more sunken, and a complete encircling cell ring is usually present.

Seeds

The seed has not been hitherto described. A few good mature seeds were exposed by dissecting away the surface of a cone and a good many fragments

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[1] The 'dissections' shown in Text-fig. 3, J–L, and Pl. VIII, Fig. 12, were made by immersing the specimen in ordinary commercial paraffin (kerosine) and scratching away the matrix, or the substance of the fossil, with sharpened needles under a binocular microscope.
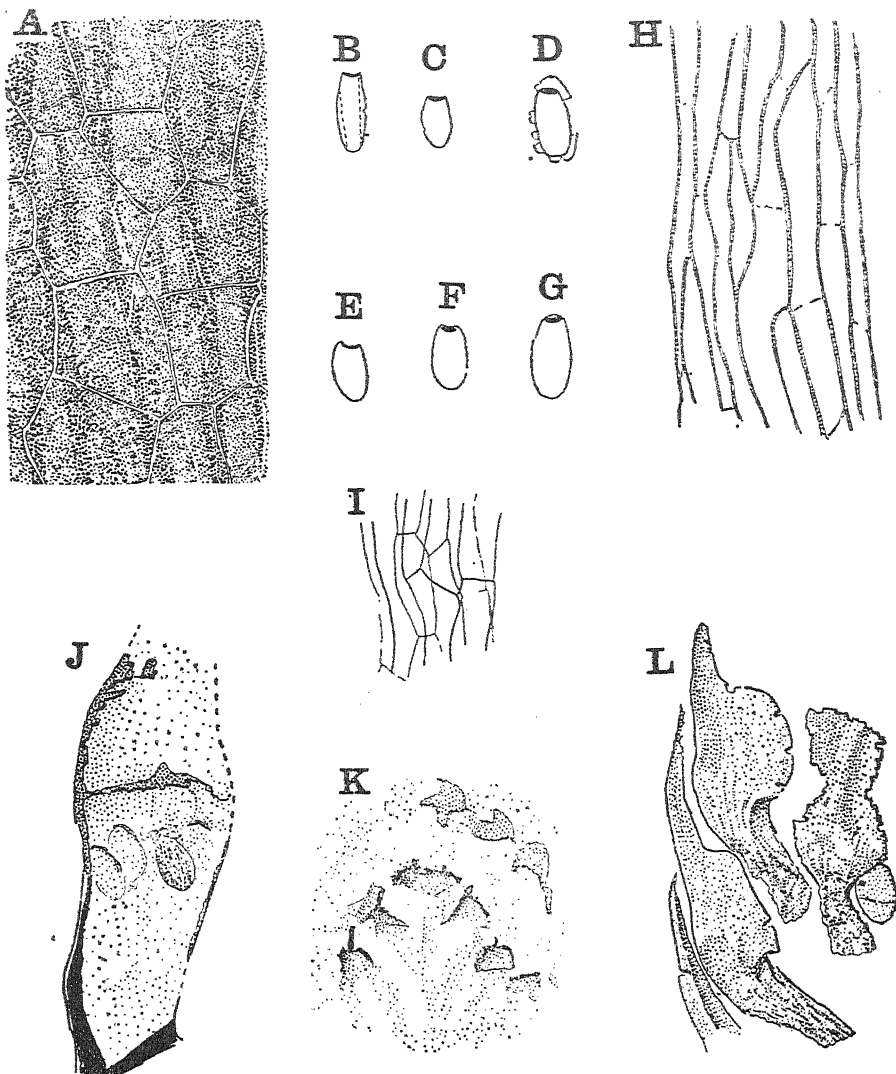
TEXT-FIG. 3. *Elatides williamsoni*, female cone and seed. A, portion of inner membranes of macerated seed, showing obscurely marked, elongated nucellar cells and clear margined cells of endosperm. V 26849 (× 400). B–G, inner membranes of seeds (all × 5). The archegonia depression at the apex is shown black; in D fragments of the integument are present. B, C are V 26850; D, H are V 26846; E–G are V 26851. H, cells of integument (× 400). I, fragment of combined cuticles of inside of integument and outside of nucellus. V 24674 (× 200). J, upper surface of cone scale showing part of the ovuliferous scale as a ridge, and below it one seed and two seed impressions. K, upper surface of cone scale showing a curved row of six placentae below and three of the ligular processes of the ovuliferous scale above. V 25860 (× 8). L, lower surface of cone scales exposed by the removal of several others, showing the expansion at the top of the ovule-bearing part. A full-sized seed is exposed by the removal of one of the scales (× 4). J, K, L, from V 28860, a specimen subsequently made into transfer.

of abortive ones were obtained by maceration of other cones. A number of isolated seeds were also found and identified by comparison with those in cones.

The seed is oval, about 2 mm. long, 1–1·4 mm. broad. It is compressed to a very thin layer (only 50 μ thick in the middle), and the margins are a good deal thinner and often bent in a way that suggests that the whole seed was originally flat and flexible (Pl. VIII, figs. 11, 13). The micropylar end may be slightly pointed and the whole surface shows the elongated epidermal cells clearly; the two surfaces seem exactly similar. Before maceration the seed looks rather like that of *Caytonia nathorsti*, but it is usually larger and more strongly striated.

On maceration, the seed yields the following structures:

1. The outer cuticle of the integument.
2. Traces of the stone of the integument.
3. Cuticle lining the micropylar canal and inside of the integument.
4. Cuticle of the outside of the nucellus.
5. An inner cell-layer, possibly of the nucellus.
6. The megaspore membrane.
7. The outer cell-layer of the endosperm.
8. Archegonia.
9. Pollen grains.

1. The cuticle of the integument is a rather thin but firm layer investing the whole seed except for the small basal hilum. It shows elongated cells with conspicuous lateral walls, but less conspicuous end walls. Near the micropyle the cells become isodiametric and their walls project strongly inwards. The end of the micropyle is closed in the specimens examined and rather thickly cutinized.

2. The stone of the integument. As usual this is dissolved on maceration and is chiefly represented by the space 100 μ wide at the margin of the seed between the cuticle of the integument and nucellus, but vestiges of narrow cells are sometimes seen. The margins of the seed though thinner than the middle part do not form a membranous wing as in some conifers.

3. Cuticle lining the integument. This cuticle is seldom well preserved. In the micropyle (Pl. VIII, fig. 20) it forms a short tube of rather thin cuticle showing small cells with conspicuous walls; near the mouth the surface of these cells bulges in a hemisphere. Just behind the mouth the micropylar canal is blocked by ingrowths from the sides, and here the cuticle is considerably thickened.

Below the micropyle this layer enlarges round the top of the nucellus and its cells become rather larger. At the level of the top of the megaspore it is joined by the nucellus cuticle and the two are inseparably adherent, and both continue to near the base of the seed. The combined membrane is exceedingly delicate, being so transparent as to be almost invisible until stained, and impossible to isolate except as minute fragments. These fragments, however, show the exceedingly finely marked outlines of two sets of cells, one set being shorter and even more faintly marked than the other (Text-fig. 3, 1).

4. Cuticle of the outside of the nucellus. Below the micropyle this cuticle forms a rather conspicuous dark mass of collapsed material in which the outlines of the cells are to a large extent obscured by the numerous folds. In this region the nucellus cuticle sometimes appears to be coarsely granular. Below the level of the top of the megaspore the nucellus ceases to be granular and becomes very delicate as it joins the inside of the integument. The combined layer only extends the very short distance of 15 μ beyond the side of the megaspore, at the sides of the seed, the original thickness of the nucellus tissue in the ripe seed.

The nucellus cuticle is readily detached from the megaspore at the apex, while at the sides of the seed it almost invariably washes away from it in preparation, despite efforts to keep it in place. It is so delicate that its presence over the surface of the megaspore is scarcely to be detected.

There is no sign of any definite pollen chamber in the nucellus, and no pollen grains were recognized as being inside the nucellus.

5. Inner cells of nucellus. The existence of this layer is inferred because of the presence of elongated cell-like marks on or in the megaspore, but it has not been isolated as a separate layer. These marks seem to represent the thick interiors of cells whose outlines are not preserved; they are ill defined but still conspicuous and give the megaspore a characteristic longitudinally striate appearance. It is possible that the marks represent the altered contents of an inner cell-layer of the nucellus; in some recent conifers cells containing brown tannin-like matter are found here which might conceivably preserve in this way. It is also possible that they represent the interior of the endosperm. They disappear after the prolonged maceration needed to separate the megaspore from the endosperm, and their position is thus doubtful.

6. The megaspore membrane is combined with layers 5 and 7 to form a robust ovate plate. It is rounded at the base, grows slightly narrower towards the top, and the top itself is flat or concave. The megaspore membrane is a non-cellular densely granular cuticle and with long continued maceration fragments of it can be separated from the endosperm. Near the top of the megaspore the combined cuticle becomes very thick and dark, possibly because of extra thickness in this layer; but in seeds which have been compressed obliquely this thick region is seen as a ring with thin cuticle or none at all in the centre where the archegonia occur.

7. The outer layer of the endosperm. This consists of large isodiametric cells with straight walls. In gently macerated seeds the lateral walls appear as fine dark lines and are inconspicuous, but with long maceration they are cleared and appear as very conspicuous cracks separating the thickened interiors of the cells. The interior substance retains a brown colour very persistently through maceration; it is either structureless or divided into blocks of varying size. This layer is nearly 20 μ thick after prolonged maceration and is probably responsible for the stiffness of the inner cuticles. The compressed seed shows the two layers of these cells pressed together, and no trace of cells or other structures was ever recognized between them unless layer 5 really

belongs here. Occasionally the cells of this layer do not extend quite to the margin of the megaspore or in parts are incompletely filled and represented by more or less isolated globular masses. It is thought that this layer represents the outermost cell-layer of the endosperm, the oily contents of which have been preserved as a resistant mass of resin, but the walls are less resistant and after maceration form open cracks. This endosperm layer is often unrecognizable or obscure in the ill-formed seeds which are retained in the ripe cones, but is always recognizable in the isolated seeds.

At the apex of the megaspore these endosperm cells become smaller and their outlines much less clear and the nucellar cells round the megaspore are more obvious, but collapsed so that at this region there is a dense, confused mass in which individual cells are hard to trace.

8. Archegonia have not hitherto been seen in seed compressions and they are not as a rule visible here. A few seeds show a dark mass just below the top of the megaspore which might represent the archegonial group (Pl. VIII, Fig. 20). One specimen in which the endosperm is well developed shows oval cavities in the endosperm at the top which irresistibly suggest the large egg cells of archegonia. Three are distinctly seen, but others may occur also (Pl. VIII, Fig. 19).

9. Pollen was recognized in a few specimens only. The grains are somewhat collapsed but agree with those of the male cone of this plant in size and general appearance. In one seed a group of these grains occurs near the mouth of the micropyle; in another seed, pollen grains occur inside the micropyle on the surface of the nucellus to one side of the mid-line. None were recognized inside the nucellus itself (though its collapsed spongy substance might not show them well even if present).

The comparative anatomy of the seed is discussed on p. 336.

COMPARATIVE MORPHOLOGY AND CLASSIFICATION

1. *Comparison with recent genera*

The sterile shoots of this conifer were first described as lycopodiaceous by Brongniart and by some authors who followed him, but later Bronn and Brongniart himself regarded it as a conifer, and this has been accepted for many years. In every feature newly brought to light in this investigation it agrees fully with typical members of the Coniferae. Its classification as a conifer is certain.

Within the Coniferae its position has been doubtful, as is reflected by its description by various authors under six different generic names. Some have supposed that it has Araucarian affinity, but others (Seward, 1900) recognized that the evidence was insufficient for classification.

Vegetative organs. The shoots resemble those of *Araucaria excelsa* in appearance and on this alone its earlier classification with the Araucarians was based. They are, however, very nearly as close to Cryptomeria, differing

merely in having stouter leaves, being intermediate between that genus and *Sequoia gigantea*.

The cuticle agrees rather closely with that of certain recent conifers, but differs in important respects from the majority of genera. It is normal in the fundamental organization of the stomata and in their being limited to certain well-defined bands; it is exceptional in their being irregularly orientated and irregularly spaced in these bands. These features are found in the Taxodiaceae, but rather seldom in other families. The variable development of encircling cells is less characteristic, but this too is met in the Taxodiaceae. The distribution of the stomatal bands on the two lower faces only is, however, unusual, for in most conifers including Taxodiaceae with thick leaves, they occur on the upper sides as well as the lower or on the upper sides only. This difference seems of only secondary importance and it should be remembered that it has not yet been shown to be general for Elatides.

While the characters of the foliage thus support the inclusion of Elatides in the Taxodiaceae, this is not at present pressed because the comparison should be based on fossils as well as on the recent members of the conifer families. The relevant facts about the fossil conifers are not available because only the part of Florin's work (1931) on the conifer cuticles dealing with fundamentals and the details of the living genera has yet appeared. For this reason, too, it is useless to discuss whether the leaf form and cuticle is relatively primitive in the Taxodiaceae, but to me it seems rather unspecialized.

The male cone agrees more closely with that of the Taxodiaceae than any other group. It is typically taxodiaceous in the long-stalked, partly peltate sporophyll and in possessing more than two pollen sacs. In their longitudinal attachment to the stalk and to one another it is unlike most of the Taxodiaceae and resembles the pines and podocarps, though in no other respects does it approach those families. The genus Sciadopitys of the Taxodiaceae does, however, show similar united pollen sacs. The round pollen grains are like those of many other conifers including the Taxodiaceae, while the details of the pollen-sac walls agree with nearly all genera except Araucaria where they are more massive.

The statement that the pollen sacs are united to one another and the sporophyll stalk is based on indirect but strong evidence. There is a general view that where free and united sporangia both occur in a group, the united condition is derived; but there is no convincing evidence of this in the Coniferae. If, however, it is correct, it would follow that Elatides is more advanced than, say, Cunninghamia and is thus not ancestral to it.

The female cone, taxonomically the most important organ in the Coniferae, is well developed showing that it belongs to the major group of the 'Pinaceae', and in the formation of the cone scale it agrees with the Taxodiaceae alone. In that family the spirally placed cone scales consist of almost completely concrescent bract and ovuliferous scales and bear several basally attached seeds. As in several genera of the Taxodiaceae the seeds are small and flat and their micropyles face the cone axis. The relation between the free parts

of the ovuliferous and bract scales is varied in the Taxodiaceae, but there is close agreement with Cunninghamia in which the bract scale is large and forms the surface of the cone, while the free part of the ovuliferous scale is a short but wide membrane somewhat divided into lobes or ligular outgrowths which are present also in Elatides. In both genera, too, the placentae are prominent. Points of difference which may not be very important are that in Cunninghamia there are typically three and in Elatides probably five seeds, and in Cunninghamia one or both margins of the scale are often bent back over the seeds. No other genus of the Taxodiaceae has cone scales of quite this form.

While there is no agreement in spite of much work on comparative anatomy about the primitive nature of the conifer cone scale, it is obvious that the cone scale of Elatides has exactly the same evolutionary level as the similar one of Cunninghamia.

The seed also agrees closely with that of Cunninghamia, but the importance of this cannot well be assessed because there has been no study of recent Gymnosperm seeds from the point of view of their comparison with fossil compressions. It is small and flat with a basal hilum and apical micropyle closed at maturity, in these features resembling the seeds of various Taxodiaceae. The existence of a cuticle covering the nucellus to its base and a corresponding one lining the integument shows that in this seed the nucellus is free. Comparison with recent gymnosperm seeds including Cunninghamia shows that such cuticles only occur on the surface of the epidermal layers of these two parts, and at the base of the free part of the nucellus its epidermis bends outwards and becomes the epidermis lining the integument. Below this level there is no nucellar epidermis and no cuticle, nor indeed would one be expected when the manner of growth of a seed nucellus and its integument is considered. The adhesion of the opposite cuticles of the nucellus and integument lining is normal in fossil seeds, and indeed occurs wherever cuticles are pressed together with no insulating layer.

There is nothing unusual in the megaspore membrane, and the archegonia occur where they would be expected—in a group below the depressed apex. The cells of the outer layer of the endosperm are, however, unusual. Seeds of several recent conifers were examined by maceration and sections without showing anything which would seem likely to preserve in this way; the endosperm is a massive tissue of homogeneous, soft, oil-filled cells. In Cunninghamia, however, the surface layer of the endosperm has a cutinized outer membrane showing cells like those of Elatides, and it might well be that this membrane would persist and become impregnated with fat after the inner cells had liquefied through rotting. In Cunninghamia, too, there is a layer of somewhat cutinized and resistant thick-walled nucellar cells outside the megaspore membrane which resemble the indistinct elongated cells of Elatides.

The conclusion is drawn that the seed of Elatides has much and possibly all important points in common with Cunninghamia and differs more or less

from the seeds of certain other conifers, but it is not claimed that Cunninghamia is unique in its agreement.

There is no need to list the differences between Elatides and the recent conifers of other families, except perhaps to point out that the numerous and free seeds distinguish it at once from the Araucarians. Its position as a member of the Taxodiaceae with which it agrees in all known organs is clear and within this group it is nearest Cunninghamia, though approaching Cryptomeria in foliage and Sciadopitys in the fused microsporangia.

2. *Comparison with other fossils*

Seward (1919) gives references to many conifers with fairly similar shoots which have been described under several generic names, but which are too inadequately known to be worth discussing here. Of greater interest is the comparison with *Palissya sternbergi*, a Rhaetic conifer known from shoots and female cones (see Nathorst, 1878, p. 28, 1886, p. 107; Harris, 1935, p. 78). In the course of the present investigation I realized I had also described a very similar but isolated male cone associated with *P. sternbergi* as *Amydrostrobus groenlandicus* and a very similar but isolated seed as *Chitospermum stereococcus* (Harris, 1935, pp. 148, 134). Preparations were re-examined and it was found that with prolonged maceration the 'Chitospermum' seeds showed endosperm cells exactly like those of Elatides. The same cells were also demonstrated in the shrivelled seeds extracted from *Palissya* cones. I am now therefore convinced that 'Chitospermum' belongs to *Palissya sternbergi*, but no proof that Amydrostrobus belongs to it has yet been obtained.

In my account of the cone scale of *P. sternbergi* I reported the presence of a single median seed and accordingly placed it in Sahni and Rao's genus Ontheodendron, and this may be correct; but I·now think that it may be only the median one of a group the rest of which are accidentally missing; the cone of *P. sternbergi* needs to be dissected in the same way as Elatides. It is indeed possible that *P. sternbergi* is not only allied to *E. williamsoni* but belongs to the same genus. The main interest of this would be that it would make Elatides which is at present the oldest known member of the Taxodiaceae considerably older still.

SUMMARY

1. The Middle Jurassic Conifer *Elatides williamsoni* Brongn. is redescribed.

2. The foliage is shown to have characters shared by recent genera of the Taxodiaceae and some other Conifers.

3. The microsporophylls are shown to bear three pollen sacs united to the stalk and to one another as in Sciadopitys. The pollen grain is round.

4. The scale of the female cone bears a minute lobed outgrowth on the upper side above the seeds, and a curved row of about five detachable seeds with micropyles facing the cone axis.

5. The small flat seeds agree in several respects with those of Cunninghamia.

6. It is concluded that *Elatides williamsoni* is certainly a member of the Taxodiaceae and is nearest to Cunninghamia in several respects but not ancestral to it. It is possible that *Palissya sternbergi* (Rhaetic) is a still earlier representative of the same group.

ACKNOWLEDGEMENTS

It is a pleasure to express my thanks to the Director of Kew Gardens and to the Professor of Botany at Cambridge for material of recent Conifers and to Dr. B. Colson and to Mr. W. N. Edwards for their painstaking help with the manuscript of this paper.

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EXPLANATION OF PLATE VIII

Illustrating Prof. Harris's article on 'The Fossil Conifer Elatides williamsoni'.
All the figures except 1–9 and 19 are untouched photographs taken by Mr. L. C. Willis.

Fig. 1. Male cone showing surface of sporophylls at the top and cone axis in the middle, immersed in oil. V 21409 (×4).
Fig. 2. Oblique L.S. through unripe male cone. V 26844 (×10).
Fig. 3. Head of sporophyll seen from within, opening of one of sporangia. V 26842 (×20).
Fig. 4. Part of specimen shown in Fig. 1. (×10).
Fig. 5. Shoot bearing very young male cones. V 23954 (×2).
Fig. 6. Shoot bearing slightly older male cones than those seen in Fig. 5. V 21409 (×2).
Fig. 7. Group of six nearly mature male cones. V 21411 (×2).
Fig. 8. Cone axis and sporophyll. V 26842 (×20).
Fig. 9. Laterally compressed sporophyll. V 26843 (×20).
Fig. 10. Impression of cone in the matrix with the free points of the scales exposed.

Specimen immersed in oil. (A male cone of *E. williamsoni* lies above and to the left of it.) V 24712 (× 1).

Fig. 11. Isolated seed; the apex and base are present but the thin margins have chipped off. V 26845 (× 20).

Fig. 12. Three of the 'ligular outgrowths' of the ovuliferous scale and fragments of the placentae exposed by dissection. V 25860 (× 10).

Fig. 13. Isolated seed showing the bent margins. The apex has broken off. V 26848 (× 20).

Fig. 14. Inner membranes of macerated seed showing the dark area at the apex. V 26847 (× 20).

Fig. 15. Female cone in which the apices of the scales have broken off. Specimen immersed in oil. V 39313 (× 1).

Fig. 16. Apex of seed after maceration, showing the top of the inner membranes enclosed in the integument cuticle. V 26846 (× 50).

Fig. 17. Axis of female cone in transfer (the sporophylls broke away in preparation). V 25860 (× 2).

Fig. 18. Female cone; part of the surface has been dissected away to expose the cone axis. Specimen immersed in oil. V 25860 (× 1).

Fig. 19. Apex of megaspore showing oval gaps in the endosperm resembling eggs of archegonia. Drawing on photograph. V 24674 (× 100).

Fig. 20. Apex of macerated seed after removal of the outer cuticle of the integument, showing the micropylar canal and a dark area below the top of the megaspore. V 24674 (× 100).
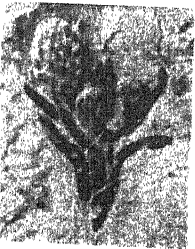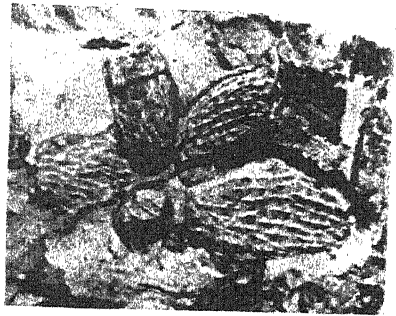
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Structure and Mechanism of the Air Pores of Fegatella (Conocephalus) conica

BY

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(*Department of Biology, University College of Swansea*)

With Two Figures in the Text

DURING an investigation into the method of water conduction in *Fegatella conica* a question arose as to whether any functional significance is to be attributed to the distribution of the two different types of air-pores—the simple, conical or dome-shaped pores on the vegetative thallus and the barrel-shaped pores with two apertures on the sexual receptacle.

The dorsal surface of the thallus has the well-known polygonal areoles which define the boundaries of the air-chambers below, each areole having at its centre a simple dome-shaped pore. From the raised pore in the centre each areole slopes downwards towards its margin, so that the boundaries of the areoles form a network of channels below the level of the pores. Moreover, the wings of the thallus also slope downwards away from the slightly raised midrib.

The flow of water was followed by brushing the surface with water and watching its course. It quickly drained off from the midrib to the margins of the wings via the channels bordering the areoles.

The elevation of the pores above the surface of the channels would therefore tend to prevent the entry of water into the air-chambers (Kny, 1890; Cavers, 1904), and this would also be hindered by the structure of the pores. The cellulose wall of the uppermost tier of cells is extended into a thin but wide membrane narrowing the opening into the air-chamber and having upon it a deposit of wax or resin granules (Kamerling, 1897). Application of Schultze's and of Schweitzer's reagent verified the constitution of the membrane as cellulose, while treatment with alcohol dissolved the granules and alkanin stained them dark red, indicating their resin nature. This narrowing of the actual opening and the complexity of the associated wall will probably be a further hindrance to the entry of water into the air-chambers.

When sections cut respectively at right angles and parallel to the surface of the fresh thallus were treated with 5 per cent. potassium nitrate or sodium chloride no effect on the pores was observed. When similar sections of fresh plants and of plants preserved in alcohol were treated with pure glycerine no movement of the pores comparable to that observed by Walker and Pennington (1939) for *Preissia quadrata* was visible. Sections mounted dry of plants

kept in a dry atmosphere also showed no variation in form of the pore. The conclusion must therefore be reached that the dome-shaped pores on the vegetative part of the thallus of *Fegatella conica* remain permanently open.

Plants were then treated on the lines adopted by Kamerling (1897), i.e. they were placed in Petri dishes containing 0·5 per cent. potassium ferricyanide and left for 2 days. Sections were then treated with alcohol to precipitate the salt and mounted in a solution of ferrous sulphate. The greatest deposit of dark blue precipitate was found in the pointed terminal cells of the assimilating filaments in the air-chambers which were immediately underneath the pores, the other cells of the filaments showing very little precipitate. Similar results were obtained by placing plants in 0·5 per cent. ferric chloride for 12 hours, mounting the sections in ammonium sulphide and noting the concentration of the black precipitate. The evaporation of water therefore appears to be localized in the pointed cells, inducing there a concentration of the contained salts. Thus the pores, though permanently open, are so arranged as to prevent water from entering the air-chambers and at the same time allow for an interchange of gases and water vapour.

The characteristic areoles of the vegetative part of the thallus are absent from the sexual receptacles, and the pores are neither so prominent nor so abundant. Further, their maximum diameter is less than that of the pores on the vegetative thallus. The outer aperture of the barrel-shaped pore is wider than is the inner one, while the walls of the uppermost tier of cells are extended in a manner very similar to that already described for the dome-shaped pores. The cells of the basal tier forming the inner aperture are, however, larger and more spherical in form, the walls bordering the aperture itself remaining thin; the whole corresponds in some degree to the 'motor' cells of *Preissia quadrata* (Walker and Pennington, 1939).

Owing to its size and convenience of handling the carpocephalum head was used for further investigation of these pores. Sections thick enough to contain complete air-pores were cut parallel to the surface of fresh carpocephala. They were mounted in water, some with the epidermis uppermost to show the outer aperture of the pore and others reversed to reveal the inner aperture and the so-called 'motor' cells; the air bubbles in the pores were removed by gentle pressure. Microscopic investigation showed both apertures to be open to their fullest extent. When, however, other sections were similarly mounted in 5 per cent. potassium nitrate or sodium chloride, although the outer, i.e. upper, aperture remained unaltered, there was a distinct diminution in the size of the lower one. On transferring these sections to water and leaving for 10 minutes the lower aperture regained its original size. Examination of sections cut at right angles to the surface showed that the large, almost spherical 'motor' cells lost their rounded contour when treated with the salt solutions and became shrunken and flaccid, causing the lower aperture to narrow appreciably. This effect was more noticeable with potassium nitrate than with sodium chloride. Treatment with alcohol caused the partial or complete collapse of the lower aperture but no change whatever was observed

in the uppermost tier of cells, the outer aperture remaining permanently open.

Other sections, both parallel and perpendicular to the surface of the carpocephala of both fresh and preserved material, were mounted in pure glycerine as described by Walker and Pennington (1939) in their experiments on *Preissia quadrata*. Five minutes after the addition of the glycerine the inner apertures
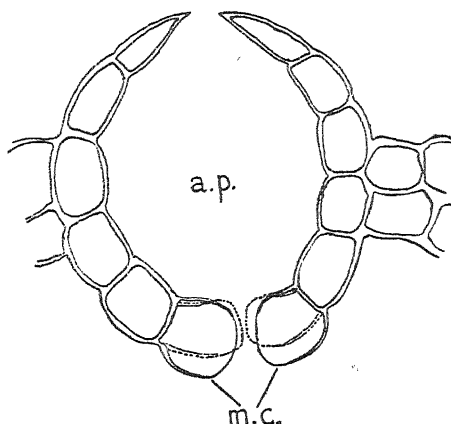
FIG. 1. Longitudinal section of barrel-shaped pore of carpocephalum. The dotted cells show the position of the 'motor' cells after treatment with glycerine. M.C., motor cell, A.P., air pore. (× 310.)

of the pores had diminished to half their normal size. However, no papillae similar to those described in the pores of Preissia could be seen, nor did the closing pores show a stellate aperture in surface view, but in vertical section the 'motor' cells appeared flatter in shape, with a greater convexity of the walls bordering the lower aperture so diminishing its size (Fig. 1). The outer aperture of the barrel-shaped pore thus behaves exactly like the simple dome-shaped pore of the vegetative thallus, while the inner aperture is capable of certain limited opening and closing movements by virtue of its thin-walled, basal 'motor' cells.

Lastly, freshly picked carpocephala were allowed to dry gradually at room temperature, and sections then cut and mounted dry. In vertical section the 'motor' cells appeared flatter than normal and irregular in shape resulting in the partial closure of the pore (Fig. 2). Recovery to normal occurred slowly when the sections were again supplied with water.

Experimental work on the method of water conduction of this species (Clee, 1943) has shown that the sexual receptacles and the sexual organs which they bear are supplied with water in exactly the same way as in *Fimbriaria bleumeana* (Bowen, 1935) and *Pellia epiphylla* (Clee, 1939), the supply to the receptacles of *Fegatella conica* being facilitated by the fact that they are sunk in grooves in the thallus. Under normal conditions the receptacles are covered by a continuous film of liquid which ensures both the liberation of the sperms

and the medium for their migration. It has already been shown that the thallus is capable of self-drainage by means of channels between the raised pores, thus avoiding waterlogging of the air-chambers; while the fact that the pores remain permanently open is not a serious drawback in a thallus which lives normally on the soil surface under conditions which involve no risk of drought. But the sexual receptacles are devoid of drainage channels, a fact
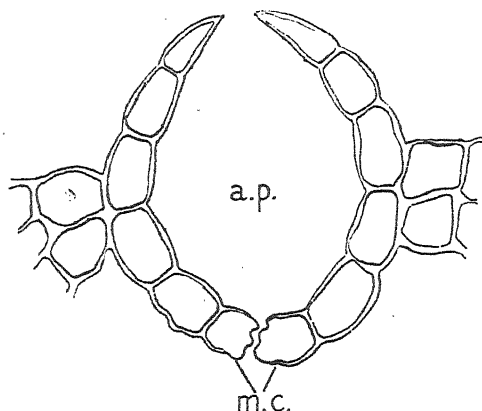
Fig. 2. Longitudinal section showing the effect of dry conditions on the 'motor' cells of a compound pore. (× 310.)

which renders the more protuberant type of barrel-shaped pore a more effective means of avoiding waterlogging than would be the more shallow dome-shaped pore of the vegetative thallus. The risk of such waterlogging would occur only while the carpocephalum is sessile. At maturation it is elevated to a height of some 6 cm. above the surface of the thallus, in a new environment in which drying of the tissues plays an important part in the dehiscence of the capsule and dispersal of the spores. This drying would clearly involve danger to the vegetative tissues of the carpocephalum, and it is readily conceivable that under these conditions the regulatory mechanism provided by the basal cells of the pore, which so alter in shape on withdrawal of water as to markedly reduce the aperture of the inner orifice, is of significance in cutting down loss of water by transpiration.

Walker and Pennington (1939) draw attention to the fact that Goebel (1882), Kamerling (1897), and Cavers (1904) all maintain that the closure of the pores does limit transpiration in situations which sometimes become 'dry', but that they give no experimental evidence in support of their statements. Walker and Pennington (1939), however, carried out weighing experiments on *Preissia quadrata* which support the view that the closing of the pores is likely to cause some diminution in the rate of transpiration under natural conditions. They express doubt, however, as to the usefulness of this power of regulating the aperture in environmental conditions which are comparatively static. The author would agree with this conclusion in relation to a prostate thallus

growing in a humid atmosphere at the soil surface, but regards the regulatory pores as of much greater importance in carpocephala raised well above the soil surface.

SUMMARY

1. The upper or ventral surface of the vegetative thallus of *Fegatella conica* is provided with a network of channels forming a simple but effective drainage system.

2. The simple dome-shaped pores of the thallus are permanently open; the compound barrel-shaped pores of the sexual receptacles have an outer aperture, which also is immovable, and an inner aperture which is capable of regulation in size by the enlargement or shrinkage of the lowest or 'motor' tier of cells.

3. When the carpocephalum head is raised into a drier atmosphere than that found at the soil surface the 'motor' cells shrink and so diminish the size of the pore, thus probably diminishing the rate of water-loss from the vegetative part of the raised head.

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The Life-history of an Organism causing Hypertrophy of the Leaf Bases of Triglochin maritimum

BY

W. R. IVIMEY COOK

AND

B. CLEAL

With Plate IX and one Figure in the Text

INTRODUCTION

IN September 1941 plants of *Triglochin maritimum* showing hypertrophy were received from the Royal Botanic Gardens, Kew. The material had been collected by Mr. John Staley from salt marshes south of Farlington Hall, near Portsmouth. Later more material similarly diseased was sent us from the Portcreek end of the Farlington–Drayton Marshes.

Preliminary examination showed that the large colourless galls occurring on the plants were caused by the swelling of the leaf bases, and sections of the hypertrophied region showed that the cells were filled with irregularly disposed masses of rounded spores.

The diseased plants were stated to occur chiefly in the wetter parts of the marshes, while those on the drier parts were free from disease. Isolated plants of *T. palustre* also occur in these marshes, but none was found exhibiting symptoms of the disease. It was concluded therefore that the disease was specific to *T. maritimum*.

The only recorded disease in *T. maritimum*, in any way resembling the present organism, is *Tetramyxa Triglochinis* of Molliard, which he discovered in France in 1909. Since, however, our knowledge of that species is very imperfect, it seemed desirable to investigate the organism more closely.

LIFE-HISTORY OF THE ORGANISM

The following description is based upon sections of the galled material fixed in Bouin's fluid, cut at 6 μ and stained in Heidenhain's haematoxylin. It was not found possible to carry out experiments on living material.

The mature galls are colourless, some are as much as 3 cm. long by 2 cm. broad, and oval in shape. The basal part of the gall bears the roots, while the apical part is more acute and passes into normal linear green leaves (see Text-fig. p. 348). The gall is sheathed on the outside by the outer leaf bases, which

are thin and papery, white or occasionally brown in colour, and entirely devoid of the parasite.

Hypertrophy is due to extensive increase in tissue of certain of the leaf bases, mainly those lying between the outer thin ones and the immature innermost ones. None of the apical embryonic tissue in the centre of the gall is infected. All the other leaf bases are uniformally hypertrophied, giving a smooth outline to the gall, although in the younger stages the parasite itself may be quite local in distribution.

The hypertrophied areas are composed of enlarged parenchymatous cells without intercellular spaces. Storage starch is often present in those cells of the gall which do not as yet contain the parasite. The nuclei of the host cells are always enlarged and when near the parasite deformed as well. The chromatin occurs in granules around the periphery of the nucleus forming patches on the nuclear membrane and a large hyaline vacuole occupies the centre. Though these nuclei may in some instances be capable of mitotic division during early stages of parasitism, they become increasingly distorted and finally disorganized as the organism develops. This condition is similar to what Nawaschin (1899) has described in *Plasmodiophora Brassicae.*

Triglochin maritimum showing the gall produced on the leaf bases by *Plasmodiophora Triglochinis.*

In their uninucleate state the myxamoebae are oval or spindle-shaped, with rather hyaline cytoplasm. The nucleus has a clearly defined nuclear membrane which is lined by a thin layer of chromatin; there is a central, hyaline, non-staining vacuole containing a single karyosome. The nuclei measure $1\cdot6-2\cdot8\,\mu$ in diameter. These myxamoebae increase rapidly in size until they finally form multinucleate plasmodia, which more or less fill the entire host cell.

At this stage, when the host cell is exhausted, the plasmodia fragment into uninucleate parts, each being surrounded by a very thin membrane. These bodies are roughly spherical and each measures about $5\cdot4\,\mu$ in diameter (Pl. IX, Fig. 4). Superficially these bodies resemble spores, and it is possible that they were mistaken by Molliard for his spores. They separate and from them are formed narrow spindle-shaped schizonts averaging $9\,\mu$ in length and $2\cdot7\,\mu$ in breadth (Pl. IX, Fig. 1). Schizonts of this kind have been reported to occur in this organism by Molliard (1909) and by Maire and Tison (1911). In very young galls the fragmenting plasmodia occur at the centre of the infected area, but the characteristic schizonts which they produce are later found all round the periphery of the infected area, adhering to the

walls of the host cells. This zonation, while not rigid, is quite characteristic and suggests that the organism spreads in this uninucleate schizont condition. Though no direct evidence could be obtained to show that these schizonts are capable of penetrating the host cell-walls, it is interesting to note that one trinucleate myxamoeba was found lying in the middle lamella between two cells (Pl. IX, Fig. 6).

These schizonts, when they reach a fresh cell, proceed to grow, and many instances were found in which a single schizont occurred in a host cell. Whether they were thus distributed by their own power or by the division of the host cell, could not be satisfactorily determined, but there is no doubt that the spread of the organism through the gall is largely due to schizont formation. During subsequent growth they lose their fusiform shape and, if more than one occurs in the cell, the cytoplasm coalesces to form a single multi-nucleate plasmodium (Pl. IX, Fig. 2). By growth this plasmodium ultimately fills the greater part of the host cell. Probably schizont formation may then take place again and thus the spread of the organism is further increased (Pl. IX, Fig. 3).

Meanwhile the older plasmodia in the centre of the gall continue their development. Up to now the cytoplasm has been fairly uniform in appearance throughout the plasmodium, but now cleavage furrows occur, separating the mass into uninucleate portions, and this is quickly followed by the deposition of cellulose in the furrows, cutting up the plasmodium into uninucleate cells. This condition, therefore, is somewhat similar to the formation of the zoosporangia in Ligniera (Cook, 1928).

At the same time as these occurrences in the cytoplasm changes occur in the nuclei. Chromatin, both from the peripheral lining layer and also from the karyosome, is extruded into the cytoplasm and the position of the nuclei is marked by transparent vacuoles. This akaryote stage has been recorded in all species of the Plasmodiophoraceae which have been investigated cytologically and is found invariably to precede spore formation. In fact it may be re-garded as an early stage in the prophase of the heterotypic nuclear division (Pl. IX, Figs. 11–12), for the chromatin reappears in such nuclei in the form of a synaptic knot. Two nuclear divisions now follow one another in rapid succes-sion. In the second the metaphase plate is clearly smaller than in the first and there is no reason to doubt that these two divisions represent a meiosis prior to spore formation.

The behaviour of the organism at this stage differs from all the other species in that the formation of the walls precedes and not follows the division of the nuclei (Pl. IX, Fig. 8). Not only is the cytoplasm divided into uninucle-ate portions at the beginning of the reduction division, but walls again appear at the end of the heterotypic division so that at the end of the homotypic division the walls of the separate spores are already partly formed.

The spores themselves are spherical bodies, though at first they appear hexagonal by the nature of their wall formation (Pl. IX, Fig. 17). When mature they measure from $5 \cdot 3 \mu$ to $7 \cdot 4 \mu$ (average $6 \cdot 6 \mu$) in diameter. The

spores are scattered irregularly in the host cells, loosing contact with one another at an early stage, concurrent with the assumption of a spherical shape. Each spore possesses a thin smooth wall of cellulose and a single central nucleus. There appears to be no karyosome in this nucleus. In a mature gall large areas of the central part are completely filled with masses of spores.

Soon after this stage has been reached cellular break-down of the host tissue begins and the gall becomes disorganized, rotting sets in, and the whole mass breaks down so that the spores finally reach the soil. Undoubtedly the germination of the spores takes place only in the soil.

Several attempts were made to germinate the spores, but without success. It seems likely that some time must elapse before germination can take place. Unfortunately no facilities existed at the present time for the cultivation of seedlings of *T. maritimum* with a view to investigating the infection of fresh plants. It seems probable that the initial infection takes place through the root-hairs, as has been shown to occur in other species. In very young galls it was repeatedly observed that several separate centres of infection appeared to be developing simultaneously. This suggests that, after the initial infection of the plant, some form of multiplication occurs which ensures that the developing gall shall be well supplied with the organism. It is suggested that this is probably achieved by means of zoospores, derived from a zoosporangium, formed in the root-hairs. Such a stage was originally described in *Plasmodiophora Brassicae* (Cook, 1932) and was later confirmed by Ledingham (1934), who also found a similar stage in the life-history of *Spongospora subterranea* (1935). Such a stage would easily explain the distribution of the organism in the galls which has already been commented upon.

CYTOLOGY

The nuclear divisions during the development of the plasmodium are protomitotic and agree in all essential respects with that already described in most other species (Pl. IX, Fig. 7). It is not proposed here to enter again into the debatable question of whether the chromatin ring, produced at the metaphase, is in fact composed of separate chromosomes or not. There is no evidence in this organism to suggest that separate chromosomes are formed, but it must be admitted that the number of good nuclear configurations of this stage, found in the slides, was disappointingly small. Nor are the nuclei as large as those in certain other species. All nuclei in the plasmodium divide together and the whole sequence leading up to the akaryote stage is completely similar to that described by the senior author in a number of other species (Pl. IX, Figs. 8–10).

The meiosis is typical, with a clearly marked, relatively large heterotypic spindle (Pl. IX, Fig. 13) and a much smaller, somewhat bent homotypic one (Pl. IX, Fig. 16). There is recognizable beading of the metaphase plate and in polar view it seems evident that separate chromosomes are present. The number is probably relatively large, possibly eight or even twelve. Measure-

ments show that the chromatin mass at the metaphase of the heterotypic division is about 2·4 μ, while at the corresponding stage in the homotypic division it is only 1·2 μ. The spindles themselves are also markedly different in size (Pl. IX, Figs. 13 and 15). The heterotypic spindle is about 4 μ in length, while that of the homotypic spindle is about 2 μ.

DISCUSSION

A brief survey of the accounts of this organism will illustrate the present state of our knowledge. In 1909 Molliard observed deformation and twisting of the flower spikes of *Triglochin palustre* at St. Nectaire, Puy-de-Dôme, in France. He found that the deformation was caused by a member of the Plasmodiophoraceae, which he thought produced its spores in groups of four. In the autumn of the same year Maire and Tison (1911) found similarly deformed flower spikes in the estuary of the Orne, at Sallenelles and later in several other localities, but in this instance it was *Triglochin maritimum* which was attacked. Thus, while Molliard found that, when plants of both species were growing together, it was *T. palustre* which was infected, *T. maritimum* remaining healthy, Maire and Tison found the organism only on the latter host plant.

A study of the organism was made by Maire and Tison (1911), and this enabled them to confirm the description given by Molliard. Through the assistance of Dr. E. Pinoy they were able to obtain co-type slides of the organism described by Molliard and to compare it with their own. The two were apparently identical.

Maire and Tison found no spores in their material and were unable to recognize any spores in the co-type slide from Molliard's material. They felt therefore that there was no justification for Molliard to place the organism in the genus Tetramyxa, which had been constituted by Gobel to include his *Tetramyxa parasitica*, in which the spores were always grouped in fours, more especially as they did not believe that Molliard had ever really seen the spores in his preparations. They therefore renamed the organism *Molliardia Triglochinis* (Moll.) Maire and Tison.

This new genus therefore was constituted on the negative character that either it did not produce spores or, if it did, they had not so far been found.

In 1926 the same characteristic twisting of the flower spikes of *T. maritimum* was found by the senior author near Gravesend, Kent. On examination the cells proved to contain plasmodia and schizonts similar to those described by Maire and Tison, but again no spores were discovered. Co-type slides of *Molliardia Triglochinis* were sent to the senior author by Dr. René Maire and were carefully compared with the Gravesend material, and the author was satisfied that the two were the same.

Criticizing the genus Molliardia the senior author pointed out (Cook, 1933) that this genus was made on the absence of any spores, but that a study of such material as was available suggested that the life-cycle could only be completed by the production of spores of some kind. He held, therefore, that

it was only a matter of time before the spores would be discovered. When they had been discovered it would be time enough to decide the systematic position of the organism. Meanwhile there seemed every reason why Molliard's original name might stand, for it might well be that spores arranged in groups of four would be discovered, as Molliard thought.

The discovery of this new material therefore seemed to offer the required opportunity to clear up the question, but actually this proved more difficult than had been expected. With the exception of one small gall, found by Maire and Tison, on a single leaf of *Triglochin maritimum*, all the other examples had consisted of twisted flower spikes; in fact Molliard reported that the flowers on such spikes were sterile, and similar conclusions were formed by Maire and Tison, and from a study of the Gravesend material. On the other hand, this new material consisted of large galls, so deforming the plants that most of them failed to produce any flower spikes at all. No such deformation was seen on any of the Gravesend plants, and it is inconceivable that Molliard and Maire and Tison could have failed to notice them had they occurred.

It would seem, therefore, that in this instance a totally different pathological effect had been produced, compared with anything previously recorded. Moreover, Molliard definitely stated that he only found the organism on *T. palustre*, while nearby plants of *T. maritimum* were unaffected. In the present instance the reverse was invariably the case. Maire and Tison found their material only on *T. maritimum*, though they did not definitely state if *T. palustre* occurred in the same locality. At Gravesend only *T. maritimum* occurred in the locality where the diseased plants were found.

Turning to the question of the spores, Maire and Tison satisfied themselves from a study of co-type slides that no spores occurred in Molliard's material and that the so-called spores which he described were artifacts of fixation. Since that time no spores have been found in any material collected. In the present material the spores are the most characteristic stage and large quantities of scattered spores fill a considerable proportion of the cells of the galls.

The separation of the genera of the Plasmodiophoraceae has always been based on the arrangement of the spores, for that is the easiest and most characteristic difference between them. Unfortunately in recent years some writers with only a limited knowledge of the group have attempted to simplify the classification by uniting certain genera together. This policy has much confused the issue, for there can be only two ways of treating the species. Either full attention must be paid to the different spore arrangements and full importance attached to any clear difference, as evidence of a separate genus, or all the species must be lumped together in a single genus irrespective of spore arrangement. There can be no intermediate position. The suggestion made by one mycologist that the genus Ligniera should be sunk in Sorosphaera on the grounds that Schwartz originally described *L. Junci* as a species of Sorosphaera indicates an incomplete appreciation of these genera, while the suggestion that Sorodiscus should also be included, either in the genus Soro-

sphaera or in the genus Plasmodiophora, made by another writer, seems to be explained only on the grounds that he had never seen any of the species and did not understand anything about them.

In deciding, therefore, how to treat the present material, two questions have to be settled, firstly whether the difference in the pathology of the host is sufficient to separate the present organism from that described by Molliard and Maire and Tison, and, if not, whether there is any justification for retaining the genus Molliardia. It seems highly undesirable to the authors to continue to perpetuate this incompletely described organism of Molliard as a separate species merely because the form of hypertrophy is different, there being no obvious difference in the life-cycle so far as it was known to those authors. Hence it is held that the present organism, causing hypertrophy of the leaf bases of *T. maritimum* is the same as that found by Molliard producing twisting and sterility of the flowers of *T. palustre*, and by Maire and Tison in *T. maritimum*.

On the other hand, the arrangement of the spores is irregular, they form no ball or mass of definite shape; they are simply scattered freely in the host cells. Hence the organism cannot be included in the genus Tetramyxa, which is characterized by having the spores grouped in fours. To modify Maire and Tison's genus Molliardia, however, to include indefinitely arranged spores, would make that genus indistinguishable from Plasmodiophora. In fact, it is only the formation of walls at an unusually early stage in spore formation, which distinguishes this organism from the species of Plasmodiophora. In all other respects there is nothing to justify separating this species from the genus Plasmodiophora.

It would appear therefore in the light of our present knowledge that Molliard's *Tetramyxa Triglochinis* should now be placed in the genus Plasmodiophora under the name *Plasmodiophora Triglochinis* (Moll.) Cook & Cleal, a modified Latin diagnosis of which is appended.

Plasmodiophora Triglochinis (Moll.) Cook & Cleal: nov. comb.

Sporae angulosae et sphaeriales, 6·6 μ in diam. Plasmodia plena cellulis matricis. Schizontes subglobosi vel fusiformi. Cystes ovales et magnae, 3 cms. longae et 2 cms. diam. in foliorum basibus, aliquando in inflorescentia curvata et distorta. *Hab.* in *Triglochin maritimo* et *T. palustri* in Gallia et Britannia.

SUMMARY

1. Material of a member of the Plasmodiophoraceae has been found in southern England, causing swelling of the leaf bases of *Triglochin maritimum*, thereby producing oval galls up to 3 cm in length.

2. The life-history of the organism has been investigated and found to agree in all essential respects with that of a typical member of the Plasmodiophoraceae.

3. It is considered that the organism is identical with the incompletely

described *Tetramyxa Triglochinis* of Molliard. This conclusion is supported by the study of co-type slides.

4. Since the spores are not arranged in the groups of four which are characteristic of the genus Tetramyxa, but are scattered in an indefinite manner in the host cells, a character of the genus Plasmodiophora, it is proposed to transfer the organism to the latter genus, under the name *Plasmodiophora Triglochinis* (Moll.) Cook & Cleal, *nov. comb.*; a modified Latin diagnosis of this is given.

5. This species differs from all the other members of the family in that wall formation accompanies the meiosis prior to spore formation instead of beginning after the completion of the homotypic division as in all other species so far as is known.

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EXPLANATION OF PLATE IX

Illustrating Dr. W. R. I. Cook's and Mr. B. Cleal's article on 'The Life-History of an Organism causing Hypertrophy of the Leaf Bases of *Triglochin maritimum*.'

All drawings were made with a camera lucida at table level with a tube length of 150 mm., using a Bausch and Lomb 2 mm. (n. a. 1. 30) objective and Zeiss compensating ocular × 15, giving a magnification of 2,500. They have been reproduced without reduction.

Fig. 1. Typical schizonts after migration, adhering to the wall of a host cell situated at the periphery of the infected area.

Fig. 2. Older schizonts in process of becoming myxamoebae and developing into mature plasmodia.

Fig. 3. A large myxamoeba which has fragmented into uninucleate schizonts, some of which have assumed the spindle-shaped form.

Fig. 4. Cyst formation, showing considerable variation in size. The nuclei are atypical with a peripheral aggregation of chromatin and no karyosome.

Fig. 5. Cysts with varying numbers of nuclei, which show the aggregation of chromatin in two areas inside the nuclear membrane.

Fig. 6. A trinucleate myxamoeba lying between the cellulose walls of two adjacent cells. The middle lamella has broken down.

Fig. 7. Stages in protomitosis in a typical plasmodium. Unlike most examples the component nuclei are not all at the same stage of division.

Fig. 8. Formation of walls cutting up the plasmodium into uninucleate portions. The karyosome has disappeared.

Fig. 9. Another stage of the akaryote condition with chromatin extruded into the cytoplasm, but in which the karyosome has not yet disorganized.

Fig. 10. The final akaryote stage in which the whole content of the nuclei has disappeared leaving a nuclear vacuole. The cytoplasm of the plasmodium stains more deeply due to stainable chromatin in it.

Fig. 11. The early prophase of meiosis. Chromatin already reappearing as a lining layer in the nuclei, but the cytoplasm still stains deeply.

Fig. 12. A later stage in the prophase in which the chromatin has become aggregated into knotted threads projecting into the nuclear vacuole.

Fig. 13. The heterotypic metaphase. The plate appears solid in side view but more granular in polar view. The spindle is clearly formed, with a vacuolate area around it. The nuclear membrane has disappeared.

Fig. 14. The heterotypic anaphase. A single degenerating nucleus is seen in one cell.

Fig. 15. The homotypic metaphase. Cell formation has continued so that the daughter nuclei, formed at the end of the heterotypic division, have already been surrounded by walls.

Fig. 16. The homotypic anaphase. The chromatin has separated into two groups and the spindle has become curved. The size of the chromatin mass is clearly much smaller than in the corresponding heterotypic stage.

Fig. 17. Mature spores, showing the irregular shape of the wall due to mutual pressure. The wall is smooth and relatively solid. Later a spherical outline will be assumed by each spore.

Fig. 18. An abnormal condition in which meiosis is taking place in a part of a plasmodium in which cell formation has already been completed and the cells so formed have separated from one another. This cell may be regarded as a sporangium.
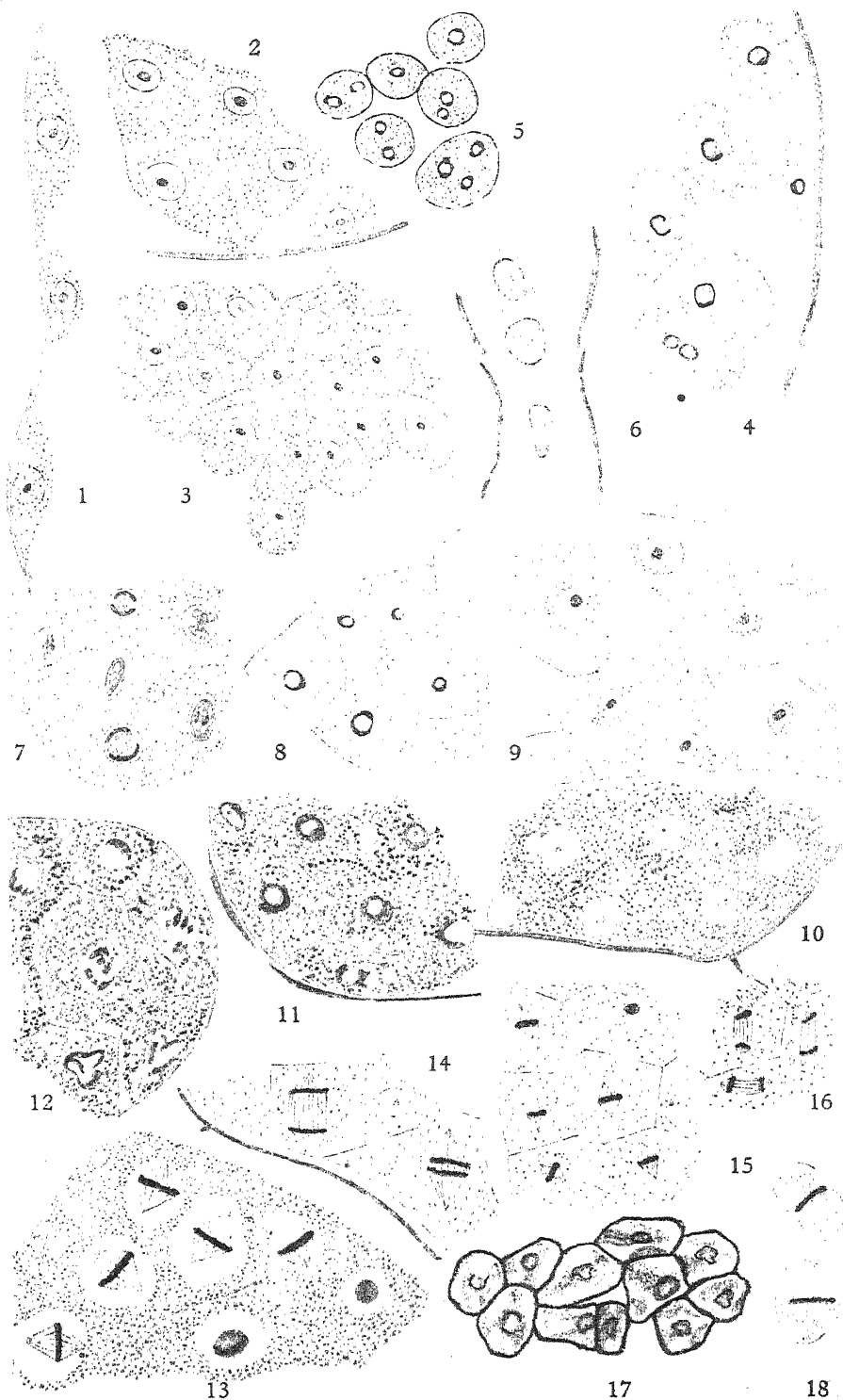
Experimental and Analytical Studies of Pteridophytes

II. Experimental Observations on the Development of Buds in Onoclea sensibilis and in Species of Dryopteris

BY

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With Plate X and twenty Figures in the Text

INTRODUCTION

THE category to which buds in certain ferns should be assigned has long remained undetermined. In view of the importance of precise knowledge of the origin and development of buds in the elucidation of problems of morphological organization and physiological integration, further investigations seem desirable. It has already been shown that plantlings which can be induced to develop on the rhizome of the ostrich fern (*Matteuccia struthiopteris* Tod) by the removal of the apex, originate from superficial areas of meristematic cells (Wardlaw, 1943). These areas, or *detached meristems*, which are present in a quiescent condition in the normal rhizome, are referable in origin to the *apical meristem*. The latter, in leptosporangiate ferns, may be precisely indicated and specified as a single superficial layer of distinctive meristematic cells, derived from the apical cell and clothing the terminal region of the apical cone. In relation to the character of the growth development, parts of the apical meristem become systematically detached and persist on the surface of the shoot in proximity to regions of meristele conjunction (in the dictyostelic vascular system) and nowhere else. From these and related observations, the conclusion was drawn that experimentally induced plantling-buds owe their origin neither to apical forking unequally developed, nor to adventitious growth, but, as indicated, do bear a definite relation to the shoot meristem. These observations not only direct attention to the importance, distinctive character, and precisely definable extent of the apical meristem in leptosporangiate ferns, but lead us to inquire whether any other ferns show detached meristems similar to those of the ostrich fern and if the origin of certain fern buds, hitherto undetermined, may not be capable of solution along similar lines. Such investigations may also lead to a more fundamental conception of the nature of branching in vascular plants. In the present paper an account is given of experimental investigations of the development of buds in *Onoclea sensibilis*, a species related to *Matteuccia struthiopteris*, and in species of Dryopteris, the genus being one which occupies a central position among leptosporangiate ferns.

BUD DEVELOPMENT IN *Onoclea sensibilis*

This fern spreads by means of dichotomizing horizontal rhizomes on which the leaves are borne (Text-fig. 1). Occasionally lateral branches of the type illustrated in Text-fig. 2 have been obtained; these are quite distinct from
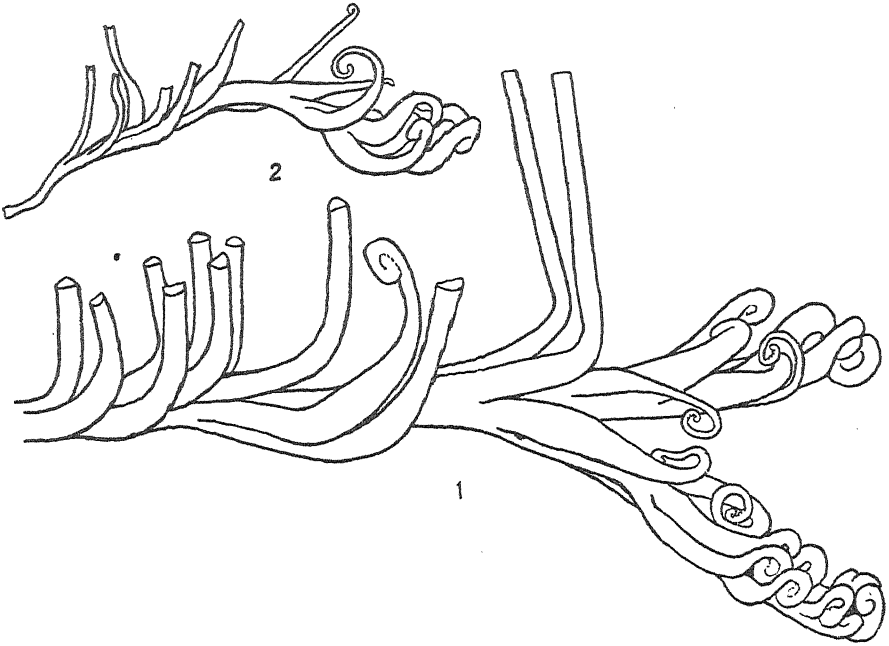
TEXT-FIGS. 1 and 2. *Onoclea sensibilis.* 1, Dichotomizing rhizome and 2, lateral branch. Roots not shown; older leaves have been trimmed off. (× 0·75.)

the shanks of an apical dichotomy. Rhizomes may appear to be quite devoid of lateral buds, but in some material small outgrowths of pale-green tissue may be observed in definite positions, i.e. at the apical ends of the conspicuous stem grooves. The further development of this bud-tissue is normally inhibited.

When pieces of decapitated rhizome are placed in peat in a cool greenhouse, or in an incubator at 22°–25° C., plantlings develop in the course of two or three weeks. The plantling-bud first appears as a hemispherical outgrowth. This elongates and may occupy an erect or semi-erect position during the development of the first one or two fleshy scale-leaves, but with the appearance of small adult leaves the shoot assumes the horizontal position characteristic of the normal adult plant. In respect of their position on the rhizome and early development, young plantlings of *O. sensibilis* bear a close resemblance to those induced on the rhizome of *M. struthiopteris* (Wardlaw, 1943). By the end of the growing season plantlings induced during early summer in a cool greenhouse have the appearance of the lateral branch illustrated in Text-fig. 2. Plantlings produced in the incubator at 22°–25° C. after growing

to some 3·0–5·0 cm. in length usually bifurcate, with equal development of the two shanks.

Both in the cool greenhouse and in the incubator rhizomes have typically
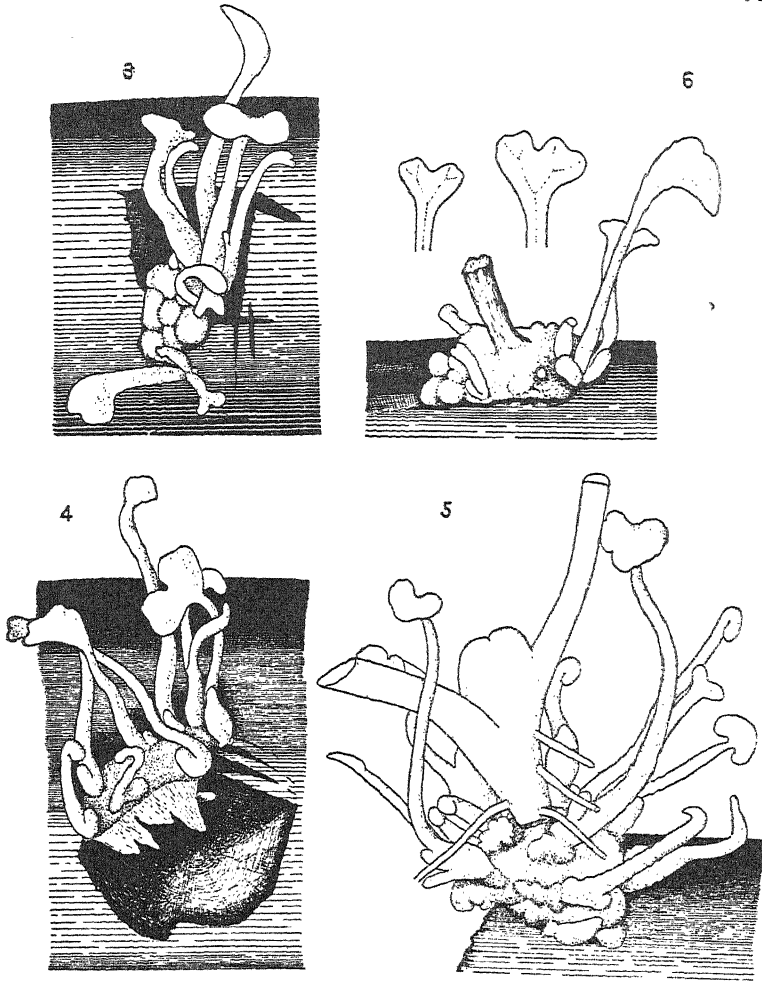
TEXT-FIGS. 3–6. *Onoclea sensibilis.* Figs. 3 and 4, numerous small buds and 'coralloid' growths developing on rhizomes from which buds have been dissected out. Figs. 5 and 6, 'coralloid' growths and numerous small buds, bearing juvenile leaves, arising round the bases of induced plantlings which have been injured. (× 9.)

yielded one strongly growing plantling, bearing small *adult* foliage leaves, at each potential formative region. Some rhizome material, however, when placed in the incubator has yielded several plantlings at each formative region, these plantlings being characterized by their small size and by the possession of *juvenile leaves.*[1] Again, in experiments where the original

[1] Environmental factors causally related to these developments may include high temperature and humidity in conjunction with relatively feeble illumination.

plantling was dissected out and the material returned to the incubator, many small plantlings and coralloid growths were produced, apparently from the cortical tissue below or laterally from the epidermis (Text-figs. 3–4 and Pl. X, Fig. 3). In yet other instances where the original induced plantling had been mechanically injured or damaged by desiccation, considerable numbers of plantlings and coralloid growths were obtained (Text-figs. 5, 6).

As in *M. struthiopteris*, the development of buds on the rhizome of *O. sensibilis* is closely related to the removal or destruction of the apical meristem, untreated controls as a rule showing no bud development, or only after a considerable time when other factors may have become operative. In a considerable number of instances which will not be treated in detail here, it was found that bud development may take place where normal apical growth has been temporarily arrested or modified as a result of experimental treatment; partial defoliation of the rhizome may also be attended by the development of plantlings.

As explained in the introduction buds which can be induced on the rhizome of the ostrich fern are referable in origin to the presence of *detached meristems*. Briefly, the development of plantlings on the rhizome of *O. sensibilis* affords a very close parallel, detached meristems being readily demonstrated in proximity to points of meristele conjunction, i.e. in the axis of a leaf but some distance above its insertion on the rhizome (Pl. X, Figs. 1, 2). The detached meristem which occupies a sunken position becomes raised and dome-shaped when formative growth begins. As in the ostrich fern these detached meristems are traceable in origin to the apical meristem. Lastly it may be noted that buds which develop in the immediate vicinity of the apical meristem (i.e. in experiments where the latter has been arrested but not damaged or removed) are in vascular continuity with the shoot stele; those induced in older regions have no such connexion. These observations support the conclusions reached in the earlier investigation (Wardlaw, 1943). Their significance is discussed in a later section.

BUD DEVELOPMENT IN DRYOPTERIS SPP.

Species of Dryopteris afford examples of ferns in which the morphological category of the buds has hitherto remained undetermined. In the account by Hofmeister (1862) of *Dryopteris filix-mas* the position, external appearance, vascular anatomy, and distribution of buds are described and figured. He refers to them as 'adventitious buds' in contradistinction to 'true forking of the punctum vegetationis', but gives no account of their mode of origin, and further remarks that *Aspidium spinulosum* (i.e. *D. spinulosa*) 'comports itself in all its parts like *Aspidium (Dryopteris) filix-mas*', but 'the adventitious buds here met with are very near the base of the stipes'. His account makes evident the difficulties that confront the investigator seeking to elucidate the origin of these buds. In *D. filix-mas* buds 'always originate on the

back of the stipes, at the place where the protuberant swelling of the latter passes off into the more slender upper portion ... such adventitious buds are formed on vigorous plants in fertile habitats at about every twelfth frond, and much more frequently in plants growing in dry situations'. Stenzel's (1861) account is of interest in that he shows that the vascular strand proceeding from the bud may join the stelar system of the leaf-base in a number of different ways. Later investigations add little to Hofmeister's original account. Bower (1923) notes that 'their constancy and similarity of position suggests comparison with certain buds of the leaf-base which results from dichotomy', but considers that each such case as that of *D. filix-mas* presents its own problem which requires separate solution. Garnet (1936) has recorded experiments in which the apical cell of a shoot of *D. filix-mas* was destroyed by puncturing it with a needle and others in which decapitation of the shoot was carried out; in the former instance it could not be definitely ascertained that any considerable number of new buds had been induced; in the latter no positive results were obtained. He was unable to show that any particular habitat or condition favoured the production of buds, but noted that whereas young plants gave no indication of bud-formation, there appeared to be a steady increase in bud-formation with age. His observation that buds usually occur in distinct zones is in contrast to Hofmeister's idea that every twelfth leaf bears a bud, but lends qualified support to the statement by Bower (1928) that most leaf-bases bear buds. Garnet has further recorded that although buds usually occur singly on the abaxial side of the petiole, they are also occasionally to be observed on the adaxial side near to one or other of the lateral ridges of the leaf-base. On rare occasions two or even three buds may occur on a single leaf-base. From anatomical data based on *Dryopteris filix-mas* Garnet came to the conclusion that buds must be formed near the apex of the shoot while the shoot tissues are still at an early stage of differentiation, but he was unable to reach a conclusion either in conformity with Hofmeister's adventitious theory or with Bower's suggestion of an apical dichotomy unequally developed.

From the foregoing it will be seen that in *D. filix-mas*, (i) the normal position of buds (on the abaxial side of the stipe but well above its point of insertion in the shoot), (ii) the variability of their occurrence in time and position, (iii) the variety in the structural relationship between bud stele, leaf-traces, and shoot stele, and (iv) the difficulties of experimental investigation, have combined to make for the present indeterminate state of knowledge.

OBSERVATIONS ON BUDS IN *Dryopteris aristata* [1]

In this species (= *D. spinulosa*, var. *dilatata* = *D. dilatata*) the semi-erect shoot may give rise to many lateral stolons or rhizomes. Some of these may appear to be definitely associated with leaf-bases, others to be situated on

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[1] This fern occurs abundantly in some districts and materials are procurable in quantity. Considerably less attention appears to have been paid to this species than to the male fern.

the free surface of the shoot and yet others to occupy indeterminate positions between shoot and leaf-base. Some rhizomes may traverse the humus for a distance of 20–25 cm. before developing into erect leafy shoots; others are much shorter; and some may grow into bulky erect shoots almost from the time of emergence from the parental stock. Many rhizomes are quite devoid of lateral buds; in some a few may be present, generally close together; and occasionally there is a profuse development of buds. These buds arise on the free surface of the shoot and not on the bases of the fleshy scale-leaves.

The effect of decapitation and defoliation on bud production may now be briefly considered. In the writer's experiments the removal of rhizome apices has been attended by the production of occasional buds and plantlings; as these have been found to be in vascular connexion with the stele of the rhizome the inference is that they were already present in a dormant or in-hibited condition, their renewed growth being related to the elimination of the dominating influence of the apex. Buds and plantlings were obtained in greater numbers when rhizomes, bearing a distal group of green foliage leaves were defoliated (Pl. X, Fig. 4), or defoliated and decapitated. Here again the anatomical evidence indicated that the experimental treatment had merely led to a renewal of growth in buds already present in an inhibited or resting condition. A conclusion based on a considerable number of experiments is that new buds cannot be induced in adult regions of the rhi-zome of this fern. The negative result of anatomical investigations to ascer-tain if detached meristems occur in *D. aristata* is in conformity with these experimental observations. Transverse sections through rhizomes bearing buds or plantlings show that almost invariably the bud stele joins the vas-cular system of the rhizome at a point of conjunction of two meristeles, the apical region of the bud being typically carried forward, i.e. towards the apex, during the longitudinal extension of the rhizome. In short, in respect of positional relationship to the parent rhizome, buds of *D. aristata* closely resemble those of *Matteuccia struthiopteris* and *Onoclea sensibilis*.

In an experiment in which the apical cell of an erect shoot was destroyed, a cluster of buds developed in the terminal region. While externally these buds appeared as outgrowths from leaf-bases, their vascular connexions with the rhizome stele were as described above. In another experiment a stout erect shoot was defoliated, only the apex and three adjacent leaf primordia being left intact. Below the apex the stock was injected with 0·25 per cent. NH_4NO_3.[1] The plant thus treated was planted in peat, the apical region being protected by means of moist cotton-wool. In the course of ten weeks the three afore-mentioned leaf primordia had developed into normal foliage leaves, a compact terminal bud of new leaves had been formed, and *immedi-ately below* the expanded leaves a ring of lateral buds had developed. A number of experiments have been carried out along these lines with comparable results, i.e. a cluster of buds has developed at approximately

[1] These experiments will be considered in detail in a later paper.

the level of the shoot apex at the outset. Microscopic examination of the specimens yielded the following observations. The new buds had developed on the bases of the leaves left undamaged at the beginning of the experiment and on damaged leaf primordia in proximity; they were not in vascular continuity with the stele of the shoot (except where they had been penetrated
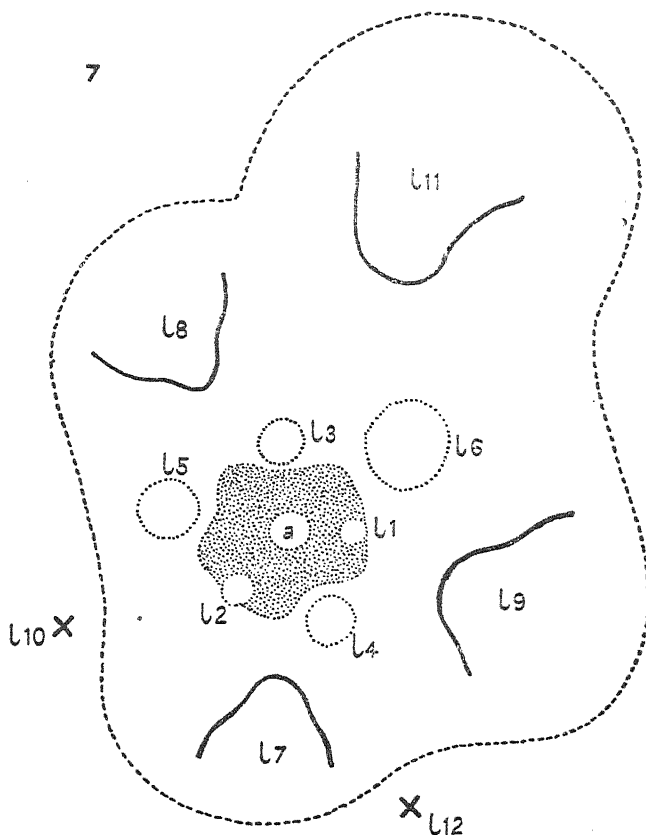
TEXT-FIG. 7. *Dryopteris aristata.* Terminal region of small erect shoot as seen from above, showing semi-diagrammatically the positions of the apical cone (*a*) and leaf primordia (*l1–l12*), the extent of the apical meristem being indicated by stippling. (× 67.)

by developing roots), i.e. the bud steles ended blindly in the outer cortex, but their positions bore the same relation to the shoot stele as already described on p. 362. Apical growth of the parental shoot had continued normally, no buds being associated with the new leaf primordia.

A specimen collected in the field in which the apex had apparently been damaged or become inhibited showed a profuse development of buds in the terminal region but nowhere else.

Collectively this evidence indicates: (i) that buds in *D. aristata* are formed in the region of the apex of the erect shoot or rhizome or they are not formed

at all; and (ii) that recognizable detached meristems are not formed or do not persist (see below).

The apical meristem. The terminal region both of the rhizome and of the erect shoot is typically of a flattened dome shape, the actual apex consisting of a small, centrally placed cone, round the base of which leaf primordia may be observed. The 'three-sided' apical cell is surrounded by a superficial layer of tissue consisting of distinctive, prismoid, meristematic cells; these have been directly derived from the apical cell and together with it constitute the actual *apical meristem.* This tissue occupies only a comparatively small area of the dome-shaped terminal region. New leaf primordia originate as a result of enlargement of individual meristematic cells near the basal margin of the apical meristem, young primordia soon being separated from the latter by the small-celled tissue of the leaf axil. In Text-fig. 7, the apex of a small plant as seen from above is illustrated diagrammatically, the extent of the apical meristem (based on an examination of longitudinal sections of comparable material) being indicated. The apical meristem of *D. filix-mas* is closely comparable.

As already stated, the examination of many apices cut in transverse and longitudinal directions has yielded no evidence of the presence of detached meristems of the type observed in the ostrich fern or *Onoclea sensibilis,* nor has the development of buds in the immediate vicinity of the apex so far been observed in material collected in the field. Again, although induced buds apparently do not originate from recognizable detached meristems, they occur in the same specific position as do normally occurring buds. It may therefore be inferred that a superficial area of tissue, lacking the distinctive appearance of meristematic cells, but possessing greater meristematic potentiality than adjacent tissue, is present in these specific positions. The experimental induction of buds at such loci will therefore involve a change in the course of development of differentiating cells which would otherwise have yielded epidermal tissue.

OBSERVATIONS ON BUDS IN *Dryopteris filix-mas*

Materials collected in Cheshire have yielded data which are in conformity with those summarized above (p. 361). In some old stocks the condition recorded by Hofmeister has been observed, namely, that a single bud occurred on about every twelfth petiole-base, i.e. approximately one bud annually. In others, buds have been found on each of two, three, or four successive petiole-bases, with groups of 8–10 bud-free leaves in between. Only as a result of experimental treatment have buds been formed on every petiole-base (see below). Petioles bearing dormant buds, on being placed in peat in a cool greenhouse, have readily yielded leafy plants; bud-free petioles under similar conditions have shown no growth development. A majority of the buds observed were of the squat, bulky type, but occasional rhizomatous specimens, up to 4 cm. long, and comparable in appearance and anatomy

with the horizontal rhizomes of *D. aristata*, have been encountered in the older regions of stocks.

In a considerable number of experiments buds have been induced to develop in the apical region of the shoot following destruction of the apex. Defoliation alone often proved effective in bud formation, the exposed apices being protected by means of moist cotton-wool. In one defoliation experiment, in which only the shoot apex and the innermost ring of leaf primordia were left intact, the following observations were made:

> Leaves 1–10: new primordia round shoot apex;
> Leaf 11: damaged;
> Leaves 12–13: undamaged;
> Leaves 14, 15, 16, &c.: removed at beginning of experiment;
> Buds: bud *A* associated *basally* with leaf 9 and *marginally* with leaf 17: buds *B* and *C*, associated with leaf 11; no buds present on any other leaf bases or associated with leaf primordia at apex.

Of these buds, *A*, though it emerged from leaf 17 in a lateral position, was found to be in vascular connexion with the shoot stele at the conjunction of meristeles basal to leaf 9. Bud *B* became conjoined with a leaf-trace of leaf 11 and bud *C* had no connexion with either shoot stele or leaf-trace, but had been penetrated by a root.

In another defoliation experiment in which the shoot apex was left intact even the smallest visible leaf primordia were destroyed by puncturing with a needle. In the course of five weeks during which the shoot was maintained in moist peat in a cool greenhouse a number of new leaf primordia had appeared and these were also destroyed. During the next five weeks there developed a terminal leafy bud, consisting of 21 leaf primordia with abundant scales; immediately below there was evidence of bud development. A majority of the new leaf primordia showed normal structure and development; a few were of small size; leaves 16, 17, and 18—the first to be formed after the experimental treatment—showed interesting anomalous developments; leaf 19 was small and necrosed; leaves 20 and 21 were apparently normal; leaves 22, 23, &c., were those which had been removed. It may be inferred that leaves 19, 20, and 21 constituted a 'whorl' of very young primordia left after the second defoliation, leaf 19 being damaged.

No buds were found in association with the new leaves, Nos. 1–21, but buds were observed as follows on the bases of excised leaves (Text-fig. 8):

> Leaves 22, 23, 24: one bud each.;
> Leaves 25, 26: no buds.;
> Leaves 28, 29: two buds each.   Total: eight buds.

Of these eight buds not one was in direct vascular connexion with the shoot stele or leaf-traces: in several instances, however, buds had been penetrated by developing roots. The buds were situated either on the leaf-base near its point of confluence with the shoot, or some distance along the petiole. At the stages of development shown in Text-fig. 8 it is difficult to assess what the initial spatial relationships of the several bud primordia to the shoot or leaf-

bases may have been. Such induction of buds may be causally related to the removal of leaf primordia, to a temporary arrest of apical activity due to the experimental treatment, or to both.

Early stages of bud-development. A considerable number of observations
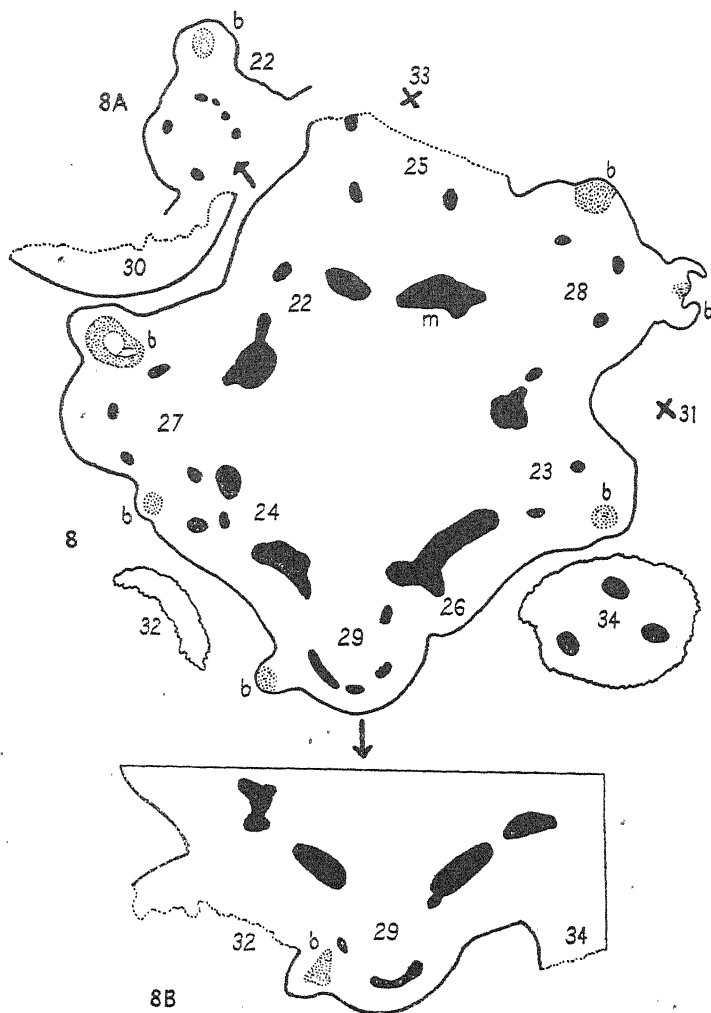
TEXT-FIG. 8. *Dryopteris filix-mas.* Transverse section of a shoot showing the positions of eight experimentally induced buds (*b*) (stippled); *m*, meristele; 22, 23, &c., leaf numbers in basipetal order. Not one of these bud steles became conjoined with the vascular system of the shoot. For further details, see text. (× 8.)

have now been made on the early stages of bud development using the following methods. From the apices of stout erect shoots all the older leaves and leaf primordia were excised and the scales removed by means of fine forceps until the apical cone and very young leaf primordia could be clearly observed under a binocular microscope, i.e. the terminal region of the shoot was

virtually 'naked'. The basal region was trimmed off, leaving a distal portion
1–2 cm. in length. Petiole buds on short lengths of petiole were also sub-
jected to similar treatment. By means of a fine needle operated directly
by hand or held in a Zeiss micromanipulator, the shoot apex and in some
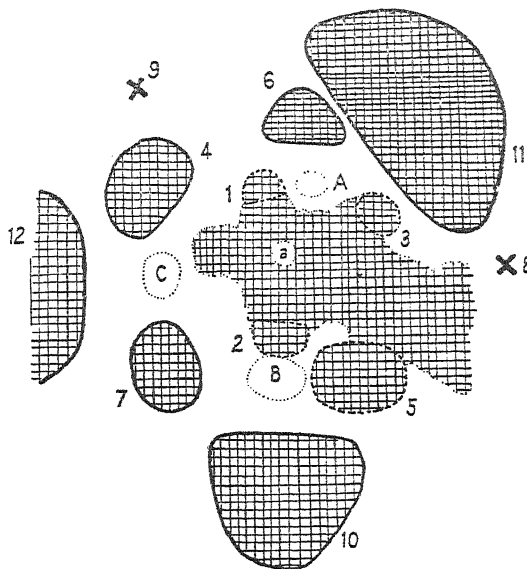
TEXT-FIG. 9. *Dryopteris filix-mas*. Terminal region of erect shoot as seen from above.
a, apical meristem; the younger leaf primordia have been destroyed; the older primordia
which have been excised are numbered 1, 2, 3, &c., in basipetal order. *A, B, C*, buds in the
axils of leaves 6, 10, and 12 respectively. Cross-shading indicates necrosed, corky tissue. (× 4.)

instances the apices of all visible leaf primordia were destroyed. The terminal
region was then covered with moist cotton-wool and the specimens placed
in moist peat in an incubator, or in a cool greenhouse, and examined after
an interval of 10–14 days. During this time a profuse development of scales
usually took place near the apex and round the leaf-bases. These scales were
carefully removed and sooner or later evidence of bud formation was obtained.
The materials were then fixed for sectioning.

The evidence from many experimental observations may now be briefly set
out and illustrated. It has been conclusively demonstrated that, *notwith-
standing the eventual abaxial position of buds some distance along petiole bases,
each bud at the time of its formation occupies a position on the shoot which is
approximately axillary.*[1] They originate superficially in close proximity to
points of meristele conjunction in the vascular meshwork of the shoot, and
therefore, in respect of their initial morphological and spatial relationships,
are closely comparable with those of the ostrich fern and *Onoclea sensibilis*.

[1] The term *axillary* is not absolutely correct: the buds do not occupy the leaf axil proper
but emerge from the shoot just above it. No other adequately descriptive term is available.

Text-fig. 9 shows the positions of three buds in the terminal region of a shoot as seen from above; bud *A* is axillary to leaf 6, bud *B* to leaf 10, and bud
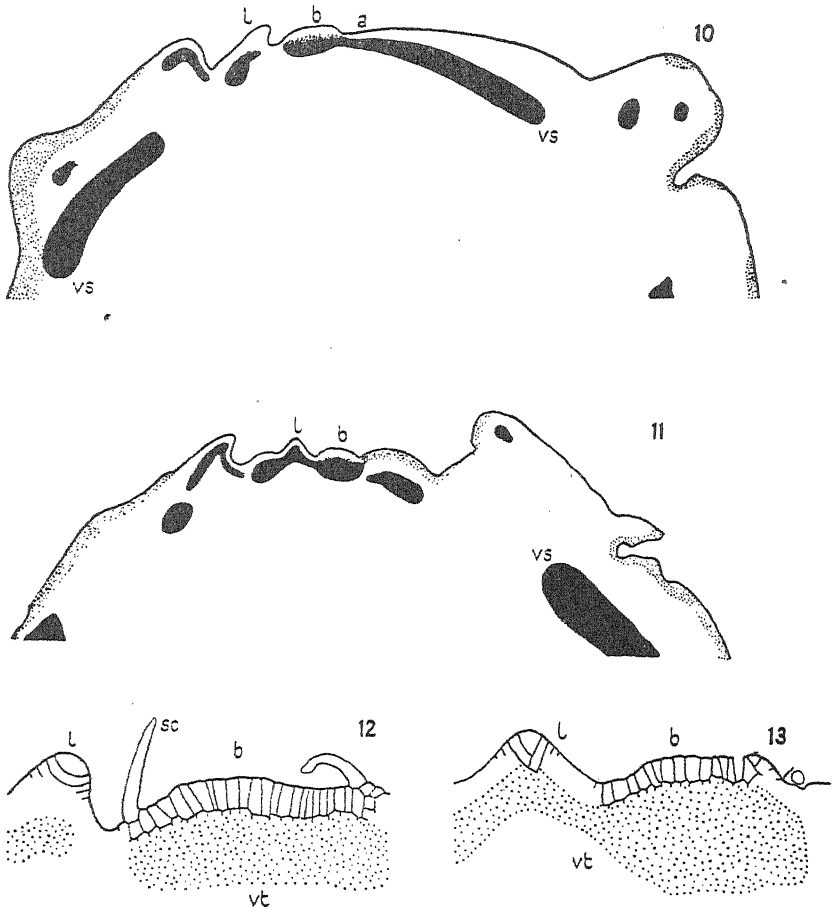
TEXT-FIGS. 10–13. *Dryopteris filix-mas.* Fig. 10, a nearly median longitudinal section of a shoot apex with an induced bud (*b*) situated in the axil of a leaf primordium (*l*); *a*, position of apex, not seen in this serial section; *vs*, vascular strand. Fig. 11, a slightly more tangential section than Fig. 10, on the opposite side of the apical cell, showing an induced bud (*b*) lying in a lateral position to leaf (*l*); had development been allowed to continue this bud would probably have been carried on the base of leaf (*l*). Figs. 12 and 13 indicate the distinctive meristematic tissue of the two buds shown in Text-figs. 10 and 11 respectively; they overlie vascular tissue (*vt*) (stippled) in the initial phase of differentiation. In neither bud had an apical cell yet become apparent; *sc*, scale. Text-figs. 10, 11 (× 14); Text-figs. 12, 13 (× 63).

C to leaf 12. Text-figs. 10, 11 show two longitudinal, approximately median sections of a shoot, treated as described above, these being located on opposite sides of the damaged meristem. In Text-fig. 10 leaf (*l*) is seen in radial longitudinal section and bud (*b*) lies in its axil; in Text-fig. 11 leaf (*l*) is seen in tangential section and bud (*b*) is lateral to it, but axillary to an older leaf.

In both buds a superficial layer of distinctive meristematic cells is present, but at this early stage of development no enlarged apical cell is yet apparent (Text-figs. 12 and 13 and Pl. X, Figs. 7–9). An examination of the whole series of sections shows that each of these buds overlies a position of meristele conjunction.

Induced buds which develop in close proximity to the shoot apex are in complete vascular continuity with the conducting system of the parent shoot. Where they have developed less close to the apex, various abnormal or in-
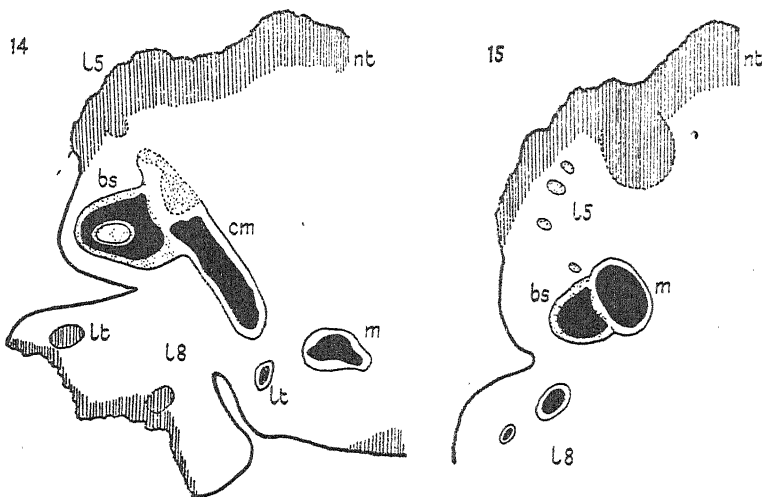
TEXT-FIGS. 14 and 15. *Dryopteris filix-mas*. Fig. 14, transverse section through an experimentally treated petiole bud showing the relation of the stele (*bs*) of an induced bud to the conjoined meristeles (*cm*) of the parent shoot (partly necrosed as indicated by stippling). This conjoined meristele occurs at the 'closing' of the leaf gap of leaf 8 (*l* 8), i.e. the induced bud lies in an obliquely axillary position to that leaf and therefore occupies a lateral position on leaf 5 (*l* 5) (partly necrosed as a result of experimental treatment). Fig. 15, transverse section through same bud taken a little lower down, showing the relation of the bud stele (*bs*) to the meristele (*m*); as in Text-fig. 114 no xylem-to-xylem connexion has been established. *nt*, necrosed tissue; *lt*, leaf-trace. (× 14.)

complete conjunctions with the vascular system of the parent shoot have been found. Thus in Text-figs. 14–15 and 16–19 it will be seen that while the vascular system of the bud becomes conjoined with the shoot stele, the xylem of the bud does not make any contact with that of the shoot. In other specimens, e.g. Text-fig. 20, a union of bud and shoot stelar tissues may be observed, but only over a limited area relative to the total area of contact between the two steles. A detailed account of these phenomena will be held over until later. Lastly, where buds have been induced in positions still further removed from the shoot apex, no vascular connexion between bud and shoot steles is effected, the bud stele ending blindly in the outer cortical tissues. Since buds originate superficially in proximity to points of meristele conjunction, and since roots emerge from the shoot stele in this region, buds are frequently found to be penetrated by roots. Whether factors are

involved other than the evident spatial ones indicated, cannot be determined on the evidence at present available.

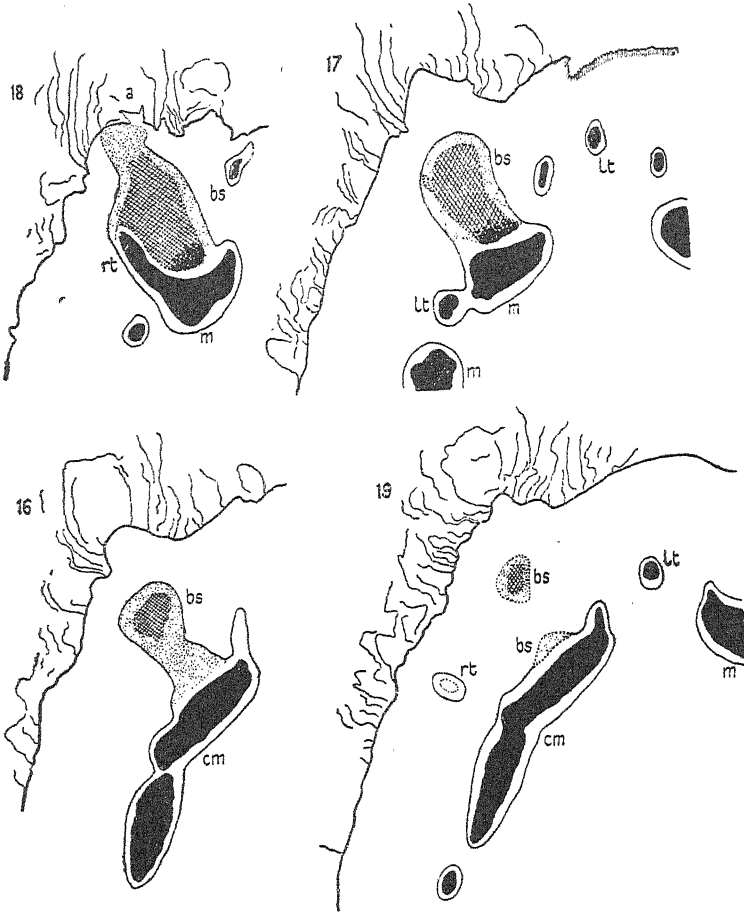In this general and introductory account of bud development in *D. filix-*

TEXT-FIGS. 16–19. *Dryopteris filix-mas.* Transverse sections through an experimentally treated petiole bud showing the position of an induced bud, with its apex (*a*) shown in Fig. 18. Figs. 17 and 18 are progressively higher in the parent shoot than Fig. 16, and Fig. 19 is a little lower. Collectively these show that the induced bud originated slightly above the point of meristele-conjunction (*cm*) and obliquely above the axil of the leaf whose foliar-gap 'closed' at that level. The junction of bud stele and shoot stele is incomplete, there being no xylem-to-xylem contact at any point. At the level of Fig. 19, the bud stele is beginning to disappear in the cortex; indications of induced histological changes associated with the development of the bud are also apparent in the conjoined meristeles (*cm*); *m*, meristele; *lt*, leaf-trace; *rt*, root-trace, intrusive into the bud in Fig. 18; bud phloem, stippled; incompletely differentiated bud-xylem, cross-shading; differentiated bud-xylem, black; endodermis is indicated by a continuous line round meristele and bud stele. (× 15.)

mas two further points must be given some consideration. These are (i) the very remarkable displacement of buds from their initial axillary position, and (ii) the histological processes involved in the inception of bud develop-

ment at positions some distance from the apex, since neither bud primordia nor detached meristems are apparently present in this fern.

Displacement of buds. In Text-fig. 9 three *axillary* buds are indicated. Now it is apparent that this is only one way of describing the positional
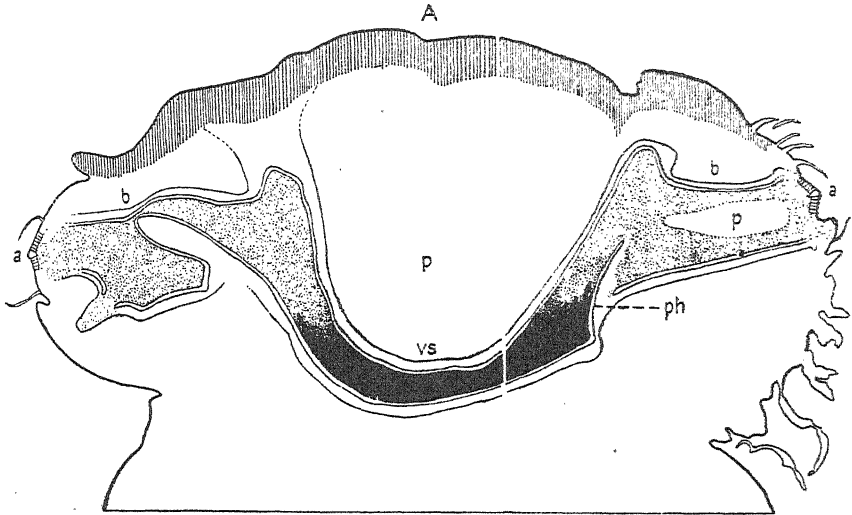
TEXT-FIG. 20. *Dryopteris filix-mas.* Petiole bud with necrosed apical region (*A*) and two lateral buds (*b*) in longitudinal section. This and adjacent serial sections indicate that the union of the bud stele with the vascular system (*vs*) of the parent shoot is not complete over the whole area of contact, e.g. note the position of the phloem (*ph*) at the union of the right-hand bud with the shoot stele. *p*, pith; *a*, apices of buds; bud-xylem and undifferentiated xylem of parent shoot, stippled. (× 17.)

relationships of these buds, since they may also be described as being basal to leaves inserted higher on the shoot and lateral to the leaves on either side. This may be further illustrated by reference to the typical stelar mesh-work present in species of Dryopteris; the individual leaf-gap is approximately hexagonal in shape and buds arise in close proximity to points of meristele conjunction, i.e. where a leaf-gap 'opens' or 'closes'. In other words, buds are essentially interfoliar. Now a feature of the developing leaf primordium of *D. filix-mas* is the very great transverse growth expansion of the basal region; this is accompanied by growth in length. There is also a forward or acropetal displacement and a lateral displacement of the superficial tissue of the shoot as it expands radially from the initial conical condition at the apex to the adult cylindrical condition. As a result of these several growth changes, a bud primordium initially occupying an interfoliar position also undergoes considerable displacement. Even in the early stages the axillary bud is displaced from the truly median position (Pl. X, Fig. 6). Buds may be carried upwards on the enlarging bases of the leaves which were lateral to them in the embryonic phase, and so become separated in space from the leaves to which they originally stood in an axillary relationship; in occasional

instances a developing leaf-base may carry up the two buds which were lateral to it during the embryonic phase. In other instances a bud may be carried up on the leaf-base to which it originally stood in a basal relationship. In the rare instances where three buds have been observed on a petiole base, it may be inferred that initially two were lateral and one basal to the leaf primordium.

This account of the displacement of buds from their initial axillary, or interfoliar position, which is in full accord with all the facts known to the writer, raises the question of the association, in the adult condition, in material collected in the field, of the bud stele with the vascular system of the petiole base, i.e. the leaf-traces, rather than with the shoot stele. Here again the explanation relates to conditions obtaining at the shoot apex; these can only be dealt with briefly here. At a very early stage the leaf primordium consists of a small papillate outgrowth of the apical meristem, its vascular system in the first phase of differentiation being solid, ellipsoidal in cross-section, centrally placed and conjoined with the shoot stele which, in the terminal region, is a continuous dome-shaped layer not interrupted by foliar gaps. As the leaf primordium develops (accompanied by growth of the shoot) it undergoes a remarkable enlargement, involving a very considerable parenchymatous development in both cortex and pith and a subdivision of the single primordial stele into 6–8 separate strands or leaf-traces; these are conjoined with the shoot stele which, in relation to these several developments, has meanwhile become perforated by hexagonal leaf-gaps. Now all the available evidence indicates that, during normal development, the formation of buds relative to the growth of leaf primordia takes place very slowly. As the differentiation of the the bud stele will be correspondingly delayed, it will therefore tend to become conjoined, not with the shoot stele, from which it has become separated in space, but with one or more of the traces of the developing leaf primordium with which it has become associated as described above.

In *D. aristata,* by contrast, even though buds may subsequently be observed in positions on petiole bases, it may be inferred that they are formed sufficiently early or develop sufficiently rapidly to permit of their steles becoming conjoined with the shoot stele. Occasionally in this species bud steles have been observed to be conjoined with leaf-traces, but these points of union have usually been close to the shoot stele. The same initial relation of bud to parent shoot is thus to be observed in both *D. filix-mas* and *D. aristata,* but differences in the distribution of growth and in the relative rates of growth of leaf and bud primordia in the two species may be held accountable for the differences in the eventual positions of buds. The influence of the apex in regulating bud development in the two species suggests interesting possibilities for further investigation.

The inception of bud development. Where buds develop in the axils of the leaves which constitute the first and second 'whorls' at the apex, each bud primordium will consist of a group of the meristematic cells which collectively

constitute the apical meristem. Many of the experimentally induced buds in *D. filix-mas*, however, have developed lower down on the shoot, i.e. in positions lying outside the apical meristem but still on the dome-shaped terminal region, and it may be that some of the buds which develop in nature likewise originate in similar positions. But whereas in *Matteuccia struthiopteris* and *Onoclea sensibilis* the development of buds in such positions or in dult regions of the shoot can be referred to the presence of detached meristems, no such distinctive tissue has been observed in either *D. aristata* or *D. filix-mas*. Nevertheless, in Dryopteris spp., at specific positions on the shoot corresponding to those in the above-mentioned ferns, but subject to the displacement already considered, the development of buds can be induced. Thus although the superficial cells in these positions resemble adjacent cells in general appearance, size, and contents (i.e. epidermal tissue in the course of differentiation), it may be inferred that they remain more highly potential in respect of formative activity. The alternative explanation that they occupy positions to which the metabolites involved in formative activities tend to diffuse most readily appears to receive less support from the observed facts. The development of buds at these specific positions under experimental treatment or 'normal' conditions thus involves a modification in the course of development of the partly differentiated epidermal and cortical tissue, i.e. what has been described as dedifferentiation must take place. Materials for relevant observations were obtained by fixing and sectioning shoot apices 10–20 days after the apical cell had been destroyed (under summer conditions in a cool greenhouse). The changes observed included successively (i) a renewal of cell division and growth at the aforementioned specific positions; (ii) the development of a distinctive superficial meristem, consisting of elongated prism-shaped cells occupying the distal region of the hemispherical bud protuberance; (iii) the appearance of a 'three-sided' apical cell; and (iv) the development of scales and leaf primordia. From stage (ii) onwards the initial differentiation of stelar tissue could usually be observed, this tissue becoming more or less completely conjoined with the differentiating shoot or leaf stele as already described. Some of these developments are illustrated in Pl. X, figs. 7–10.

As bud-formation only takes places in the terminal region and not in older or adult regions of the shoot, the inference may be drawn that there is a basipetal gradient of meristematic activity which disappears in the region of fully differentiated adult tissues.

DISCUSSION

In some ferns, e.g. Hymenophyllaceae, buds occupy definite axillary positions—the prevailing condition in flowering plants. But in many ferns they occur in positional relationships to shoot or leaf less easy to define and not apparently conforming to any generalized morphological category. In the present study it has been shown that the extra-axillary buds in Dryopteris spp. are essentially axillary in origin but that they may undergo very

considerable displacement during the growth development of the shoot and leaves. In relation to the physiological dominance of the shoot apex and other factors at present little understood, bud-formation in these ferns may be of periodic or occasional occurrence only. Buds are also characterized by a slow rate of growth, i.e. there may be little evidence of bud development until the associated leaf primordium is in the third or fourth 'whorl' removed from the apex. Thus while a bud may be initially axillary to one leaf, its position during development is essentially *interfoliar*. Buds may thus be regarded as developing from those regions of the apical meristem which have not been used in the development of leaf primordia. In attempting to understand why a bud develops and why it eventually occupies a certain position the integrated growth activities at the apex must be considered; in particular, the investigation of the relative growth of the several organs and tissues is essential to a more adequate understanding of the adult organization.

In different ferns the shoot may be short and thick with a close assemblage of leaves, or, as in rhizomatous types, elongated and with the leaves more widely separated; leaf-bases may remain relatively narrow and crescentic in cross-section or may become greatly distended and approximately circular in cross-section. According to the nature of the growth development more or less notable departures from the initial spatial relationships of organs at the apex may result, e.g., in *D. filix-mas*, the quite remarkable displacement of buds. The buds of *D. aristata* are like those of *Matteuccia struthiopteris* and *Onoclea sensibilis*; each lies in the longitudinal axis of a leaf but at some distance above the leaf axil, and may therefore be situated on the base of a leaf higher up on the shoot. Stenzel's excellent observations show that this bud-shoot relationship also obtains in other species of Dryopteris as well as in *Blechnum spicant, Athyrium filix-femina, Polypodium alpestre, Diplazium giganteum*, and *Alsophila aculeata*. Such observations suggest that in respect of their initial positional relationship to shoot and leaves the shoot buds of ferns (as distinct from the arrested shanks of dichotomies) may all prove on further examination to be fundamentally alike, the position which they eventually occupy being determined by the specific distribution of growth in shoot and leaf. Whether or not other instances of the presence of buds in extra-axillary positions, as in Cheiropleurea, Metaxya, Lophosoria, Dennstaedtia, &c., will prove explicable along the lines indicated here is still an open question; experimental studies of bud development at the apical meristem may be indicated as affording the means by which the answer may be obtained.

Bower (1923, p. 77) has indicated that some extra-axillary buds can be referred to 'an origin in dichotomy of the axis, with unequal development of the shanks, and with a close relation of a leaf to the base of the arrested shank'. But in *Onoclea sensibilis* both dichotomy of the shoot—involving presumably the equal division of the apical cell, or the rapid growth of a meristematic cell immediately adjacent to the apical cell as in *Polypodium vulgare* (Klein, 1884)—and the outgrowth of distinctive lateral buds can be found in the same specimen (Text-figs. 1, 2). In this matter Stenzel's observations go

far to clarify the position for leptosporangiate ferns, for he has pointed out that whereas in dichotomizing shoot systems there is an equal distribution of pith, vascular meshwork, and cortex between the two shanks (one of which may become inhibited and may ultimately occupy a lateral position) the distinctive lateral buds—the so-called adventitious buds—are usually conjoined with the outside of a shoot meristele by a single strand of vascular tissue, i.e. by a protostele. That these two categories of lateral bud can be readily distinguished has been verified during the present investigation. These two seemingly distinct categories of branching, however, are both referable in origin to a cell or group of cells constituting part of the apical meristem. Temporal, spatial, and physiological factors at present insufficiently investigated may be held responsible for the two different types of development observed. The plasticity of the formative region in ferns becomes increasingly evident, and until fuller information is available it appears desirable to reserve judgement on the view that branching in ferns in general and in a phyletic sense may be referred to dichotomy.

Hofmeister regarded the buds of Dryopteris spp. as being adventitious in origin. Stenzel has opposed this view on the grounds that it is 'absurd' to regard as casual or adventitious buds which always occur in definite positions. But in the case of Dryopteris buds not formed at the actual apical meristem, Hofmeister's view is in a sense correct though incomplete in important details. The present investigation has shown that 'adventitious' buds develop in specific positions corresponding to those occupied by the detached meristems in *Onoclea sensibilis* and in these positions only, the reorganization of a superficial meristem being an essential preliminary condition for bud development. It appears that partly differentiated tissue possessing meristematic potentiality persists in these positions as long as the growth enlargement of the shoot is still incomplete. This formative capacity does not persist in the adult region of the shoot.

In *Matteuccia struthiopteris, Onoclea sensibilis*, and in species of Dryopteris, buds originate superficially on the shoot in proximity to regions of meristele conjunction. These may be indicated as regions of minimal distension of the superficial tissues during growth and development. In Dryopteris spp. detached meristems do not occur; in this connexion the very great distension of superficial tissues, which necessarily accompanies the development of the large pith and cortex typical of these ferns, may perhaps be considered as being causally related to this condition. Attention is accordingly directed to the valuable data which should accrue from comparative studies of the relative growth development at the apex of different ferns, particularly with a view to obtaining a more precise understanding of the organization characteristic of the adult plant.

SUMMARY

1. In *Onoclea sensibilis* experimentally induced plantlings always arise superficially on the rhizome in proximity to points of meristele conjunction

in the dictyostelic vascular system. At these points areas of distinctive meristematic cells—described as *detached meristems*—are present in a quiescent condition in the normal rhizome. In respect of bud formation, *Onoclea sensibilis* closely resembles *Matteuccia struthiopteris* (Wardlaw, 1943).

2. In *Dryopteris aristata* buds may occur on the free surface of the shoot or on leaf bases. Except for the fact that buds tend to be carried forward, i.e. in an acropetal direction, during elongation of the shoot, the positional relationship of bud to shoot is as in *Onoclea sensibilis*.

3. In *Dryopteris filix-mas* buds typically occur on the abaxial sides of petioles but some considerable distance above the insertion of the petiole-base on the shoot. It has been conclusively demonstrated that, notwithstanding the eventual position of buds in adult regions of the plant, each bud, at the time of its formation, occupies an approximately axillary position on the shoot. In respect of their initial spatial relationships to the shoot the buds of *D. filix-mas* are closely comparable with those of *D. aristata* and *Onoclea sensibilis*.

4. The displacement of buds of *D. filix-mas* from their initial axillary position is referable to the distribution of growth in the leaf-bases and shoot. In the close assemblage of leaf primordia at the shoot apex a bud which is axillary is also interfoliar. Hence, in relation to the growth developments of leaf-bases and shoot, a bud which is initially axillary to one leaf may be carried up on the lateral flank of an adjacent leaf primordium. As a result and because bud growth is slow, the bud stele is usually found to be conjoined with a leaf-trace; in experimentally induced buds, however, the bud stele may be joined with the shoot stele, at a point of meristele conjunction as in *D. aristata*, or it may end blindly in the outer cortex.

5. In species of Dryopteris buds develop close to the shoot apex or not at all. Detached meristems, as in *Onoclea sensibilis* or the ostrich fern, have not been observed. In those instances where buds have been induced to develop at the apex, but outside the apical meristem, a reconstitution of meristematic tissue from the partly differentiated epidermal tissue takes place at specific positions, i.e. corresponding to those occupied by the detached meristems in *Onoclea sensibilis*. As such developments do not take place in adult regions of the shoot the inference may be drawn that there is a basipetal gradient of meristematic activity which disappears in the region of fully differentiated tissue.

6. These investigations indicate that in order to understand why a bud develops and why it eventually occupies a certain position on the shoot or leaf-base the integrated growth activities at the apex must be considered; the data from such investigations should eventually contribute to a fuller understanding of the factors responsible for the organization of the adult plant.

7. Comparative observations suggest that with regard to their initial positional relationship the shoot buds of ferns (dichotomous shanks excepted) may all prove to be fundamentally alike, the position which a bud eventually occupies being determined by the distribution of growth during the development of the leaf-shoot system.

The writer has pleasure in acknowledging the assistance received from Mr. E. Ashby in microscope preparations and photographic illustrations.

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EXPLANATION OF PLATE X

Illustrating Professor C. W. Wardlaw's article on the Development of Buds in *Onoclea sensibilis* and in species of Dryopteris.

All figures are from untouched photographs.

Fig. 1. *Onoclea sensibilis*. Transverse section of a rhizome showing a detached meristem, in which a small amount of growth has taken place; it occupies a position in proximity to a region of meristele conjunction. ($\times$ 100.)

Fig. 2. *Onoclea sensibilis*. Transverse section of a rhizome showing the distinctive cells of a detached meristem in the inactive condition. ($\times$ 250.)

Fig. 3. *Onoclea sensibilis*. Surface view of a rhizome showing numerous small buds which have grown out round the edge of a cavity created by the excision of the original bud. ($\times$ 5·5.)

Fig. 4. *Dryopteris aristata*. Abundant development of plantlings on a rhizome from which all leaves, except those of the terminal bud, had been removed. (Nat. size.)

Fig. 5. *Dryopteris filix-mas*. Petiole bud, with leaves removed, showing three induced buds (following destruction of apex) seen from above. The obliquely axillary position of the centre bud can be seen. ($\times$ 3.)

Fig. 6. *Dryopteris filix-mas*. Transverse section of the experimentally treated petiole bud illustrated in Fig. 5. The stele of an induced bud can be seen in relation to two conjoined meristeles of the parent shoot; it occupies an obliquely axillary position to the leaf shown on top left. ($\times$ 30.)

Fig. 7. *Dryopteris filix-mas*. An approximately median longitudinal section through the apex of an experimentally treated shoot showing an induced bud, with distinctive superficial meristematic cells, lying above the axil of the leaf primordium shown on the left. ($\times$ 125.)

Fig. 8. *Dryopteris filix-mas*. An approximately median longitudinal section through the apex of an experimentally treated shoot showing an early stage of bud development in the axil of a slightly larger leaf primordium than that shown in Fig. 7. ($\times$ 150.)

Fig. 9. *Dryopteris filix-mas*. A longitudinal section, not quite median, of an experimentally teated shoot apex, showing a leaf primordium in tangential section and an induced bud occupying a position lateral to it. ($\times$ 125.)

Fig. 10. *Dryopteris filix-mas*. Longitudinal section through a considerably older leaf primordium that those shown in Figs. 7 and 8. The apical cell of the leaf primordium was destroyed at the same time as that of the shoot. An early stage in the development of an induced bud, with nuclear division of the apical cell, is shown. The dark necrosed and corky tissue (top)lies immediately below the position of the damaged leaf apex. ($\times$ 250.)
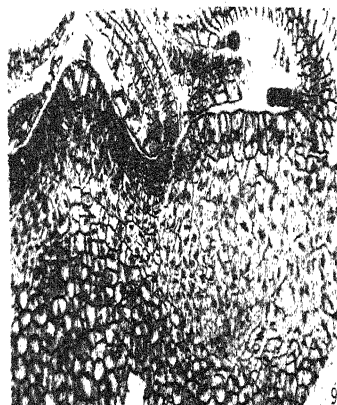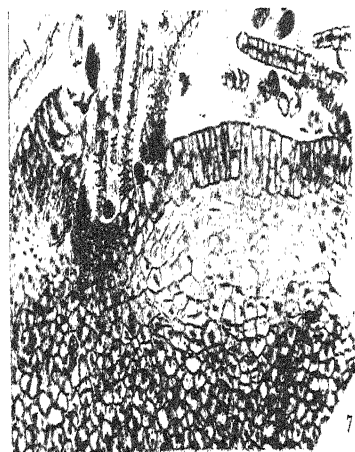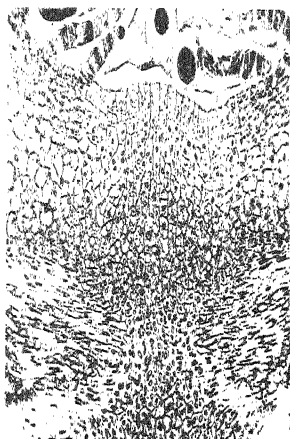
The Effect of some Purines and Related Compounds on the Seedling Growth of *Avena sativa* L.

BY

R. FORBES JONES

AND

H. G. BAKER

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With two Figures in the Text

IN the course of the work in these laboratories on the study of antigrowth substances in relation to cancer (Thompson, Holt, Jones, Kennedy, and Hajdu, 1941) extracts from human urine have been prepared. As these were tested for their effects on plant material, it was felt desirable to study the effects of known urinary constituents on plant growth. The substances selected for study were the purines and other related compounds such as guanidine carbonate, urea, &c. The extracts probably also contained auxins.

METHODS AND MATERIAL

Oats (Victory, ON 160, obtained from the National Institute of Agricultural Botany, Cambridge) were soaked in filtered, aerated tap-water for $2\frac{1}{2}$ hours at 25° C. and germinated on damp filter-paper at the same temperature for 24 hours. At the end of this period seeds with rootlets of 1–2 mm. were selected and planted in tubes of about 25–30 ml. The seeds were held in position by means of curtain netting of suitable mesh fixed across the neck of the tubes by rubber bands. Only one seed was planted per tube. Fifteen tubes were used in one small tank (with lid) containing a small quantity of water, and eight of these tanks were placed in larger tanks with lids, each larger tank also containing water. The seedlings were thus growing in an atmosphere of maximum humidity and there was little loss from the solutions in the tubes by evaporation. The temperature of the darkroom where the seedlings were grown was maintained at 25° C.±0·5.

In earlier experiments one small tank with eight tubes containing the solution to be tested and seven containing tap-water were used. Where critical results were required, as in the experiments given below, the solution to be investigated was tested, using fifteen seeds in one small tank, with a similar parallel series of water controls.

In this latter type of experiment while all the seeds were approximately of the same status in regard to germination, &c., each seed in one tank

corresponded to a seed of exactly similar status in any of the other tanks. Thus in one large tank seven test solutions with fifteen seeds each could be tested against one (or more) of water, also with fifteen seeds. Filtered, aerated tap-water was used on all occasions.

Total root length (T.R.L.) was taken 48 hours from the time of planting by laying the seedling along a glass scale, which was illuminated from below, and measuring the 4–5 roots. The average T.R.L. per seedling for each series was calculated and a percentage value obtained (T.R.L. series / T.R.L. water × 100).

Some authors (e.g. Kaiser and Albaum, 1939) have used the length of the longest root (L.R.L.) as a measure of root growth. In order to test the value of this, all the measurements obtained in these laboratories during the last three years were analysed for the relation of the L.R.L. to the T.R.L. These measurements include values obtained from two crops of oat grain (1938 and 1940).[1] The values for all the water series were collected as L.R.L. and T.R.L. for each seedling, but a relatively few seedlings which grew very slowly or were malformed have been omitted.

All the values of the test solution series from seeds of the 1940 crop were collected in a similar manner, and in these values are included those from the roots which were very strongly inhibited by active solutions and also from roots which were not inhibited by inactive solutions. The number of seeds dealt with in the 1938 water series was 4,084; in the 1940 water series 6,629; and in the 1940 test solution series 7,730.

The T.R.L. values have been selected into groups averaged to the nearest whole number in cm. and the L.R.L. values to the nearest $\frac{1}{2}$ cm. A large proportion of the seedlings have a T.R.L. of 20–2 cm., in which region lies the average generally obtained with the experimental technique employed. The mean for the 1938 water control series is 20·2 cm. and for the 1940 water control series is 20·4 cm.

On the basis of longest root length (L.R.L.) 53 per cent. of the 1938 water control series are 6·0 to 6·5 cm., and for the 1940 water control series 47 per cent. are 6·5 to 7·0 cm. The mean L.R.L. for the 1938 and 1940 water control series is 6·2 and 6·5 cm. respectively.

When the average L.R.L. for each T.R.L. group of the water control series is plotted (Fig. 1) it is seen that there is a direct linear relation between the T.R.L. and the L.R.L. In the higher T.R.L. values, however, further increase in the T.R.L. is accompanied by progressively smaller increments in the L.R.L., and there appears to be a maximum value for the L.R.L. under the experimental conditions. Increase in the T.R.L. above about 24 to 25 cm. within the 48-hour growth period must therefore almost wholly depend on the increase in length of the later developing roots.

A similar condition holds where the roots have been growing in the various

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[1] The 1939 crop gave irregular germination, as also did the crops subsequent to 1940. During the course of these experiments seeds from the 1938 and 1940 crops maintained their regularity of germination and subsequent growth.

test solutions (also see Fig. 1). Even in the instances of extreme inhibition, the L.R.L. varies directly with the T.R.L. In the same way the L.R.L. attains a maximum value at about 24 cm. T.R.L. The total numbers in the higher T.R.L. groups are relatively small, and this fact makes the L.R.L. values for these groups less reliable.

The correlation coefficients for the L.R.L. with the T.R.L. of 0·87, 0·83,
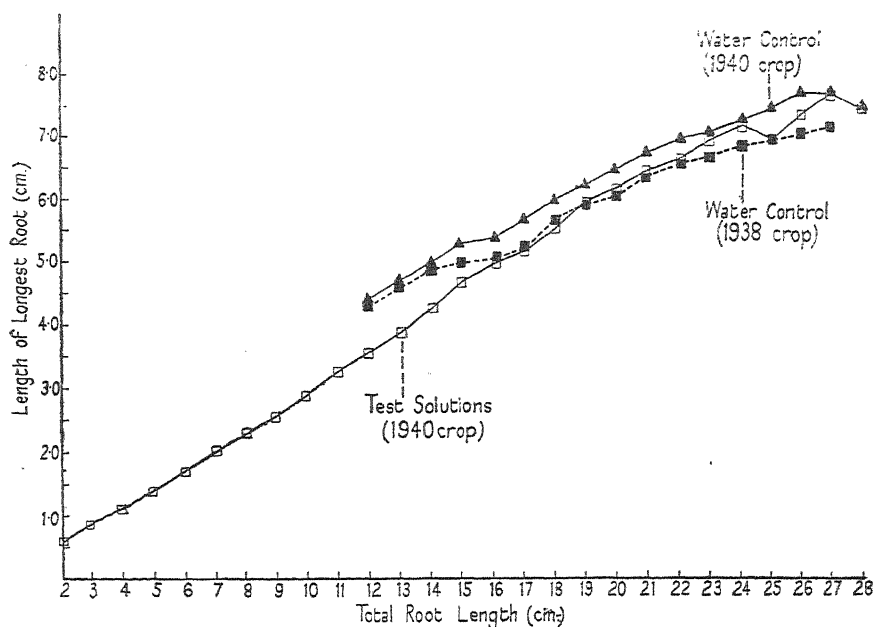
FIG. 1. Average L.R.L. for the T.R.L. of each group of seedlings in the test solutions (1940 crop) and for the water controls of the 1938 and the 1940 crops.

and 0·96 for all the 1938 water series, 1940 water series, and 1940 test solution series respectively indicate a high degree of significance in view of the thousands of seeds used. If the coefficients are calculated for the T.R.L. groups less than 22–3 cm., the value of r still more nearly approaches unity.

It might therefore be assumed that the L.R.L. is an index of the T.R.L. especially when, under these experimental conditions, the value of the T.R.L. does not exceed about 24 cm. However, when percentage growth is calculated it is found that this may differ slightly according to the basis of root length measurement. The value of percentage growth used in these laboratories is the ratio (expressed as a percentage) of the average T.R.L. in the test solutions to the average T.R.L. in water controls. On the basis of the L.R.L. this value is usually less than that calculated on the T.R.L., i.e. the inhibition appears to be slightly greater on an L.R.L. basis.

In order to test this a random batch of about a thousand results was taken. The percentage growth on a T.R.L. basis (A) and that on a L.R.L. basis (B) were calculated, and values for B/A were obtained. The value B/A

is less than unity in the majority of cases and only two were equal to unity. However, as the percentage growth on a T.R.L. basis increases (i.e. decreasing inhibition) the proportion of cases in which B/A is greater than unity increases. This is shown graphically in Fig. 2, where the percentage of seedlings per group in which B/A is greater than unity is plotted against the percentage growth on a T.R.L. basis, each group containing roughly
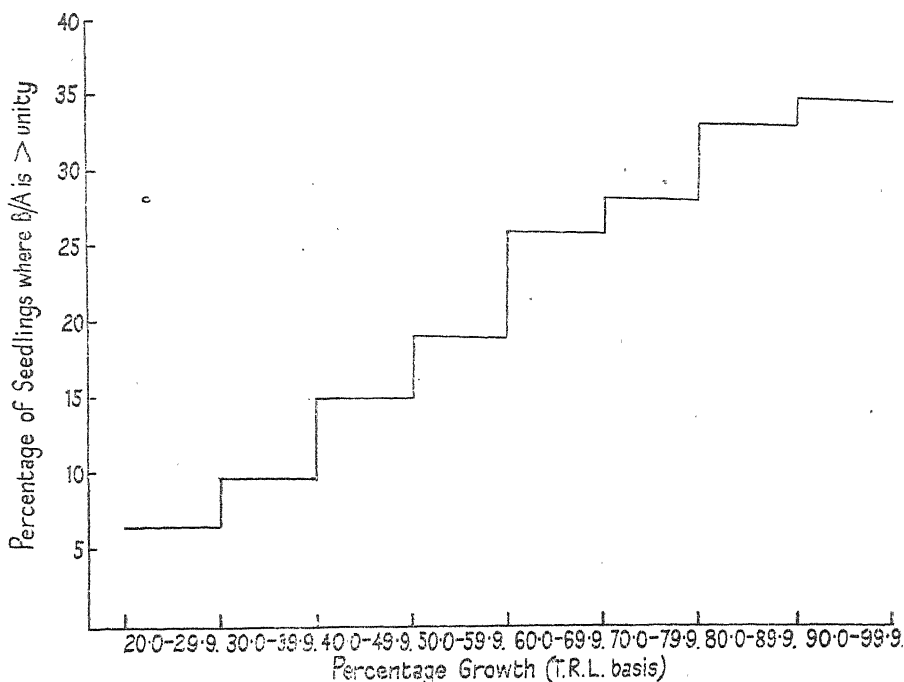
FIG. 2. For explanation see text (p. 381).

one hundred or more seedlings. It is possible that this difference according to the basis of root length measurement is an artifact caused by the angle at which side roots develop and by the relative periods of time that each of the roots may be in a solution.

It would thus appear that (1) the L.R.L. is an index of the T.R.L., there being a high degree of correlation; (2) the above is true for a wide range of the T.R.L. values obtained after 48 hours' growth, but not for the higher values where increase in the T.R.L. is not accompanied by an increase in the L.R.L.; and (3) on the L.R.L. measurements, inhibition can appear to be slightly greater when compared with values on a T.R.L. basis; it is suggested that this may be due to technical difficulties.

EXPERIMENTAL RESULTS AND DISCUSSION

The purines tested are all known to occur in the plant kingdom. In general, these are believed to be decomposed by oxidation in certain of the higher

plants, allantoin being an intermediate product of the action of uricase on uric acid. The relation between the purines in regard to their breakdown by enzymes is as follows (Bodansky, 1934):

$$\text{Adenine} \xrightarrow[\text{adenase}]{+H_2O} \text{Hypoxanthine} \quad +O \mid \text{Hypoxanthine oxidase}$$

$$\text{Guanine} \xrightarrow[\text{guanase}]{+H_2O} \text{Xanthine} \quad +O \mid \text{xanthine oxidase}$$

$$\text{Allantoin} \xleftarrow[\text{uricase}]{+H_2O+O} \text{Uric acid}$$
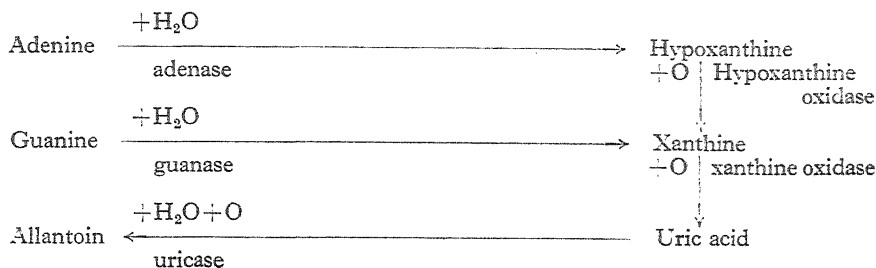
The allantoin can then be acted upon by allantoinase to yield urea and glyoxyllic acid through allantoic acid (Fosse, 1939). A further stage would be the release, from the urea, of ammonia which would then be incorporated into the protein anabolic processes.

In some anaerobic bacteria, however, e.g. *Clostridium acidi-urici*, the purine is probably oxidized by means of CO_2 which is reduced to acetic acid (Barker and Beck, 1941).

The importance of the purines in seeds and normal plant tissue is not yet clear apart from the fact that adenine and guanine are part of some of the constituent nucleotides of the complex molecule of nucleic acid. That they have an important function in the plant becomes increasingly evident from the data of such workers as Loofbourow (1942), where adenine nucleotides are shown to have a stimulating effect on yeast growth. Hypoxanthine and guanine have been claimed to be the growth factor Z_1 of Phycomyces (Robbins and Kavanagh, 1942). Factor Z_1 was obtained from potatoes. Coppin (1912) has made some observations on the effects of some purines on the growth, till flowering, of Hyacinthus, but he used only high concentrations (0·02–0·5 per cent.).

Effect on seedling root growth.

The results presented here show that the substances cause different degrees of root inhibition, while none is able to any marked degree to produce an over-all significant and constant stimulation of growth during the first 48 hours from germination. In so far as the solubilities of the different members of the series will allow comparison, adenine produced the greatest degree of inhibition in terms of molar concentration, the percentage growth at $2·0 \times 10^{-3}$ M in the experiment cited (Table I) being 29·9 per cent. (i.e. of growth in tap-water) and at $1·0 \times 10^{-3}$ M 67·3 per cent.

Another—NH_2 compound of the purine series, guanine, was soluble with difficulty and could only be tested in low concentrations. It was not always possible to retain it in solution in tap-water and results of experiments were only considered where no precipitate was formed. No growth effects of any

significance were obtained between the concentrations 1.0×10^{-4} M to 1.0×10^{-6} M, although the tendency seemed to be for a slight inhibition of the root growth to appear at the greater concentration.

The results with hypoxanthine and xanthine must be considered with the

TABLE I

Percentage Growth of Roots and Coleoptiles of A. sativa in the Dark at 25° C. in molar Concentrations of Adenine. Growth in Water = 100.

Molar concentration.	Roots.		Coleoptiles.
	T.R.L. basis.	L.R.L. basis.	
5.0×10^{-3}	14.9	11.9	48.3
2.5×10^{-3}	21.9	18.1	58.6
2.0×10^{-3}	29.9	22.9	56.6
1.75×10^{-3}	32.3	27.0	60.0
1.5×10^{-3}	42.1	35.2	63.4
1.25×10^{-3}	53.4	48.0	76.7
1.0×10^{-3}	67.3	62.9	76.7
5.0×10^{-4}	93.6	87.9	80.6
2.5×10^{-4}	98.3	97.4	86.6
1.0×10^{-4}	101.9	95.8	103.5
7.5×10^{-5}	101.0	95.8	96.3
5.0×10^{-5}	99.8	97.9	101.0
2.5×10^{-5}	99.3	97.5	98.1
1.0×10^{-5}	100.5	97.9	101.0
5.0×10^{-6}	90.9	91.6	100.0
1.0×10^{-6}	91.4	94.5	96.3

reservation that they were not tried as extensively as desired because of the difficulty of obtaining supplies under war-time conditions. That which was used was of La Roche manufacture and bought from a scientist who brought it from his laboratory in Prague.[1] However, the results indicate that hypo-xanthine even at relatively high molar concentration (2.0×10^{-3} M) was completely inactive while there was no stimulation with a dilution of 5.0×10^{-6} M. Xanthine was made more soluble by the addition of sodium hydroxide to the distilled water before making up to volume with tap-water. Under these conditions there was also no inhibition at 2.0×10^{-3} M concentration, but of all the series (except perhaps for uric acid in some experiments) xanthine was the only one to show some stimulation of the roots. This stimulation did not occur regularly with any particular concentration, but this could not be investigated further.

The effect of uric acid on root growth is peculiar in that at extremely low concentrations (5.0×10^{-5} M) a growth value of about 67 per cent. of control was obtained (Table II) and this effect could not be increased with increasing concentration to 5.0×10^{-4} M. Since uric acid could only be tried in very dilute solution it was made more soluble by neutralizing it in distilled water with sodium hydroxide before making up to volume with tap-water. The concentration was used as gram-mols of uric acid. Under these conditions the same phenomenon appeared, the value of 68–74 per cent. of control

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[1] The other members of the purine series were supplied by British Drug Houses, Ltd.

appearing between $1\cdot75\times10^{-3}$ M to $5\cdot0\times10^{-4}$ M approximately (Table III). Thus the change from inhibition to no effect occurred in the neutralized uric acid at about $5\cdot0\times10^{-4}$ M to $1\cdot0\times10^{-4}$ M, while with uric acid alone in tap-water the change occurred at $5\cdot0\times10^{-5}$ M to $1\cdot0\times10^{-5}$ M. There was no

TABLE II

Percentage Growth of Roots and Coleoptiles of A. sativa in the Dark at 25° C. in molar Concentrations of Uric Acid. Growth in Water = 100.

Molar concentration.	Roots.		Coleoptiles.
	T.R.L. basis.	L.R.L. basis.	
$5\cdot0\times10^{-4}$	66·0	69·4	82·8
$1\cdot0\times10^{-4}$	67·5	68·0	82·8
$5\cdot0\times10^{-5}$	66·9	65·3	86·2
$1\cdot0\times10^{-5}$	94·3	92·0	86·2
$7\cdot5\times10^{-6}$	95·2	91·4	81·3
$5\cdot0\times10^{-6}$	97·1	101·3	90·6
$2\cdot5\times10^{-6}$	93·3	97·1	103·1
$1\cdot0\times10^{-6}$	100·9	103·1	103·2
$5\cdot0\times10^{-7}$	98·5	103·9	100·0
$1\cdot0\times10^{-7}$	98·5	100·0	101·5

TABLE III

Percentage Growth of Roots and Coleoptiles of A. sativa in the Dark at 25° C. in molar concentrations of Uric Acid. Growth in Water = 100. (Acid neutralized in distilled water with Sodium hydroxide before making up to volume with tap-water.)

Molar concentration.	Roots.		Coleoptiles.
	T.R.L. basis.	L.R.L. basis.	
$1\cdot75\times10^{-3}$	68·4	69·7	100·0
$1\cdot5\times10^{-3}$	68·9	66·7	96·0
$1\cdot0\times10^{-3}$	71·3	72·7	104·0
$5\cdot0\times10^{-4}$	74·2	73·1	96·7
$2\cdot5\times10^{-4}$	78·8	74·6	96·7
$1\cdot0\times10^{-4}$	97·5	91·0	96·7
$5\cdot0\times10^{-5}$	97·0	92·6	96·7
$1\cdot0\times10^{-5}$	99·6	93·0	103·0

stimulation even in extreme dilution except at irregular concentrations in occasional experiments. It is not as yet possible to say what factors for growth were restricted in this limited manner by uric acid. Fosse (1939) says that uric acid in higher plants may be decomposed to allantoin by uricase and that the allantoin by allantoinase is broken down through allantoic acid to urea and glyoxylic acid. The urea is not generally found in the plant when urease is present as it would be broken down to ammonia, which might then be utilized in protein anabolism. It is certain that allantoin or urea (and, presumably, the derived ammonia) which might have been formed from uric acid in the dilutions given would not have inhibited root growth (cf. below).

Allantoin has been shown by Brunel and Echevin (1939) and Echevin and

Brunel (1939), on radish and soya bean respectively, to cause an increase in protein nitrogen. In the case of soya the presence of allantoinase makes the result understandable. In the radish, however, no allantoinase was detectable, so that the mechanism of utilization of the allantoin was unknown. In the present experiments allantoin had very little or no effect on the seedling root growth, although there was an occasional slight inhibition (70–80 per cent. of normal growth) at $3 \cdot 0 \times 10^{-3}$ M in a few of the experiments. Otherwise there was no effect as far as the lowest concentration tried ($5 \cdot 0 \times 10^{-5}$ M).

Effect on coleoptile growth.

With regard to the effect of the above purines on coleoptile growth, adenine, a leaf growth factor, caused an inhibition at $2 \cdot 0 \times 10^{-3}$ M giving a value of 56·6 per cent. of the control with the inhibitory effects disappearing at concentrations of about $5 \cdot 0 \times 10^{-4}$ M to $1 \cdot 0 \times 10^{-4}$ M (Table I). There was no significant stimulation as far as the extreme dilution of $1 \cdot 0 \times 10^{-6}$ M. Avery and Sargent (1939) found stimulation in straight growth using the method of Scheer (1939) with concentrations equivalent to $1 \cdot 5 \times 10^{-3}$ M to $6 \cdot 0 \times 10^{-7}$ M. Bonner and Bonner (1941) also found an increased production of dry matter in Cosmos and in mustard in both roots and shoots using concentrations of approximately 6×10^{-7} M to 6×10^{-9} M. The present results are in more direct disagreement with those of Avery and Sargent as when a concentration of $1 \cdot 5 \times 10^{-3}$ M was applied to the roots a significant inhibition of the coleoptile resulted, the value being 60 to 70 per cent. of the controls. Since the conditions of the experiments, period, and mode of application of the substance, presence or absence of the coleoptile tip, &c., were different in the two laboratories, an assessment of the reasons for the difference must be deferred.

However, while Avery and Sargent (1939) were able to detect a growth promotion by using hypoxanthine, a similar effect could be demonstrated under the present experimental conditions in some of the experiments. Further work would be necessary to show the significance of these results. Similarly, xanthine was able to cause a slight stimulation, though this was not always a result which could be repeated. Guanine was ineffective at the concentrations used.

As with root growth, uric acid produced a slight inhibition of coleoptile growth at the higher concentrations ($5 \cdot 0 \times 10^{-4}$ M to $5 \cdot 0 \times 10^{-6}$ M approximately); weaker concentrations showed either no effect or slight stimulation. With the uric acid neutralized in distilled water before making up to volume with tap-water, significant inhibition was not observed even at the concentration of $1 \cdot 75 \times 10^{-3}$ M. Slight stimulations in the coleoptile growth were also observed in weaker concentrations.

Although allantoin was almost without effect on root growth, there seemed to be a slight stimulation of the coleoptile growth. This stimulation was not always repeatable.

Thus, of the members of the purine series tested, adenine and uric acid are the only two which showed a significant inhibition of the root and coleoptile growth. As regards stimulation of coleoptile growth, any effect in this

TABLE IV

Percentage Growth of Roots and Coleoptiles of A. sativa in the Dark at 25° C. in molar Concentrations of Urea. Growth in Water = 100.

Molar concentration.	Roots.		Coleoptiles.
	T.R.L. basis.	L.R.L. basis.	
$3 \cdot 0 \times 10^{-1}$	45·6	47·1	70·4
$2 \cdot 5 \times 10^{-1}$	50·0	53·0	77·7
$2 \cdot 0 \times 10^{-1}$	60·7	61·7	85·2
$1 \cdot 5 \times 10^{-1}$	71·5	75·0	96·3
$1 \cdot 0 \times 10^{-1}$	81·9	80·9	100·0
$5 \cdot 0 \times 10^{-2}$	87·0	92·4	100·0
$2 \cdot 5 \times 10^{-2}$	95·5	89·5	92·6
$1 \cdot 0 \times 10^{-2}$	95·5	97·0	100·0
$5 \cdot 0 \times 10^{-3}$	99·5	100·0	103·7
$2 \cdot 5 \times 10^{-3}$	100·5	103·0	100·0
$1 \cdot 0 \times 10^{-3}$	103·4	101·5	100·0

direction was not great and was of irregular occurrence with the experimental technique employed.

A few additional substances which also are normal constituents of urine were similarly tried.

With urea, the seedling could withstand relatively high concentrations bathing the roots, and it was only at a concentration of $5 \cdot 0 \times 10^{-2}$ M that inhibition could first be detected (Table IV). At $3 \cdot 0 \times 10^{-1}$ M the inhibition of the roots was 45·6 per cent. of the control and that of the coleoptiles 70·4 per cent. There was no coleoptile inhibition or stimulation at concentrations weaker than 0·15 M.

Guanidine, which is related to urea and is a protein denaturant (Greenstein, 1939), was, as the carbonate, extremely active as an inhibitor of root growth (Table V), and this effect may be detected at a concentration of $2 \cdot 8 \times 10^{-4}$ M. The effect on the coleoptiles was also very great.

Creatine and Creatinine have both been shown by Skinner (1912) to have a beneficial effect on the growth of wheat when used in the extreme dilution of 50 p.p.m. In the experiments cited here they were seen to cause inhibitions of root growth (Tables VI and VII) at about $5 \cdot 0 \times 10^{-3}$ M and the increase of inhibition in proportion to concentration was greater in the case of creatinine, the value at $1 \cdot 0 \times 10^{-1}$ M being 29·7 per cent. of the control as against 60·0 per cent. at the same molar concentration for creatine.

Where root and coleoptile are considered together it is generally found that inhibition in the former at a concentration above a certain value is associated with an inhibition of the latter. Taken as percentages of the control values the growth inhibition of the roots is always greater than that of the coleoptiles. There may be several factors which have a bearing on the relative

TABLE V

Percentage Growth of Roots and Coleoptiles of A. sativa in the Dark at 25° C. in molar Concentrations of Guanidine carbonate. Growth in Water = 100.

Molar concentration.	Roots.		Coleoptiles.
	T.R.L. basis.	L.R.L. basis.	
1.68×10^{-2}	—	—	31·8
1.12×10^{-2}	—	—	38·4
9.80×10^{-3}	—	—	41·3
8.40×10^{-3}	—	—	43·0
7.00×10^{-3}	—	—	44·0
5.60×10^{-3}	—	—	45·6
2.80×10^{-3}	—	—	59·5
1.68×10^{-3}	29·4	27·4	77·2
1.12×10^{-3}	31·3	31·9	77·2
9.80×10^{-4}	37·9	37·7	82·8
8.40×10^{-4}	38·9	42·2	80·0
7.00×10^{-4}	46·0	47·8	85·7
5.60×10^{-4}	50·7	50·7	88·6
2.80×10^{-4}	84·9	87·0	91·5

TABLE VI

Percentage Growth of Roots and Coleoptiles of A. sativa in the Dark at 25° C. in molar Concentrations of Creatine. Growth in Water = 100.

Molar concentration.	Roots.		Coleoptiles.
	T.R.L. basis.	L.R.L. basis.	
1.0×10^{-1}	60·0	60·0	70·4
5.0×10^{-2}	62·7	64·0	81·5
2.5×10^{-2}	76·5	76·0	92·6
1.0×10^{-2}	89·2	86·7	96·3
5.0×10^{-3}	90·0	90·6	92·6
1.0×10^{-3}	95·6	97·3	107·3
5.0×10^{-4}	97·8	97·3	103·8

TABLE VII

Percentage Growth of Roots and Coleoptiles of A. sativa in the Dark at 25° C. in molar Concentrations of Creatinine. Growth in Water = 100.

Molar concentration.	Roots.		Coleoptiles.
	T.R.L. basis.	L.R.L. basis.	
1.0×10^{-1}	29·7	30·9	65·6
7.5×10^{-2}	43·6	45·6	90·6
2.5×10^{-2}	79·7	75·0	100·0
1.0×10^{-2}	87·6	85·3	96·8
5.0×10^{-3}	90·5	89·7	96·8
2.5×10^{-3}	100·0	97·0	100·0
1.0×10^{-3}	101·0	98·5	100·0
5.0×10^{-4}	99·0	98·5	100·0
2.5×10^{-4}	105·4	101·5	103·1
1.0×10^{-4}	100·0	100·0	106·1

effects on roots and coleoptiles, as for instance (*a*) the relative sensitivities of root and coleoptile to the substances, (*b*) the differences in the mechanism of growth—by cell division and elongation in the root and mainly by cell elongation in the coleoptile, (*c*) the rate of movement of the substances from

the external medium through the cells of the root to the coleoptile, and (*d*) the degree of destruction of the substances during this passage through the plant.

SUMMARY

When Avena seedlings were grown in the dark at 25° C. there was a very high degree of correlation between the total root length and the length of the longest root.

The growth of roots after treatment with various solutions was expressed as a percentage of the root growth in tap-water. This percentage growth value was generally slightly lower (i.e. inhibition appeared to be greater) when it was calculated on the longest root length basis, though this phenomenon became less marked as the percentage growth value approached 100 per cent.

Adenine, hypoxanthine, xanthine, guanine, uric acid, and allantoin were tested for their effects upon root and coleoptile growth. Adenine caused detectable inhibition of root growth at about $5 \cdot 0 \times 10^{-4}$ M, with the effect increasing with concentration.

In the case of uric acid a percentage growth value of about 67 per cent was found at $5 \cdot 0 \times 10^{-5}$ M, but this value was not altered with increasing concentration. A similar value was found with uric acid neutralized before making up to volume with tap-water, but here also at about $5 \cdot 0 \times 10^{-4}$ M the percentage growth value of about 68 per cent. could not be altered even with a concentration of $1 \cdot 75 \times 10^{-3}$ M.

There was no significant stimulation of root or coleoptile growth with any of the purines tested.

Guanidine carbonate could inhibit both root and coleoptile growth at about $5 \cdot 0 \times 10^{-4}$ M, whereas with urea no inhibition of root growth below $1 \cdot 0 \times 10^{-1}$ M was found.

Creatine and creatinine both inhibited root gowth at $5 \cdot 0 \times 10^{-3}$ M.

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Studies on Foliar Hydration in the Cotton Plant

V. A Further Experiment with Potassium[1]

BY

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With two Figures in the Text

I. INTRODUCTION

IN our first paper (Mason and Phillis, 1942) on foliar hydration, we considered the relationship between changes in potassium supply and foliar hydration. It was found with plants grown in sand culture under normal greenhouse conditions that hydration (i.e. water per 100 gm. dry weight) was more intimately related to the dry weight of the whole plant (i.e. size of plant) than to the potassium level (i.e. potassium per 100 gm. foliar dry weight). Size of plant and hydration in this type of experiment always showed a strong negative correlation. On the other hand, with plants growing in the field it was found that size of plant and foliar hydration were positively correlated as long as potassium supply was limiting dry weight production.

In potassium experiments with plants exposed to different lengths of day it was observed that hydration in long-day plants showed strong negative correlations with size, as in plants grown in sand culture, while with short-day plants, increasing potassium supply led generally to an increase in hydration, i.e. potassium supply and hydration were positively correlated.

To explain these results we suggested that where changes in potassium supply led to large changes in size, or where the environment was relatively arid, large plants would be exposed to a greater water strain than small plants and would consequently exhibit in their leaves less water per 100 gm. dry

weight than the smaller plants; hydration and *size* would therefore be negatively correlated. On the other hand, where changes in potassium supply caused only small changes in size or where the environment was relatively humid, the true potassium effect could assert itself. The true potassium effect apparently is that *potassium supply* and hydration are positively correlated.

In more recent papers on foliar hydration (Mason and Phillis, 1942*a*, 1943; Phillis and Mason, 1943) we have drawn attention to the part played by salts in cell water relations. We concluded from a study of the relation between salt concentration and hydration in the leaves of plants grown under a wide range of mineral supply that concentration was an important factor determining the level of hydration in the lamina.

Our objects in carrying out the experiment described below were (1) to investigate the effect on hydration of size under wet and dry conditions, (2) to ascertain whether potassium affects hydration by changing the salt concentration of the cell, and (3) to ascertain the relative importance of size and concentration on hydration.

In previous papers on hydration only the lamina was considered; in the present one the behaviour of the bark was also investigated.

II. PROCEDURE

The plants were grown in sand culture. Half of the plants were grown in a glasshouse which was regularly sprayed with water in order to maintain humid conditions. These plants will be referred to as the 'Wet' series. The other half grew on a platform with a mosquito wire roof. These plants will be referred to as the 'Dry' series. In each series there were six levels of potassium supply, viz. 25, 50, 200, 400, 600, and 1,000 p.p.m. Nitrogen (500 p.p.m.), phosphorus (50 p.p.m.), and calcium and magnesium (100 p.p.m each) were kept constant. There were two collections, the first when the plants were 58 days old and the second 7 days later. There were 20 plants per treatment, and these were graded at the time of the first collection into two samples of equal height, giving 10 plants at each collection. Results for water per 100 gm. dry weight and per gm. protein-N are available for the main-axis leaves and the bark. The dry weight of the whole plant (i.e. size) was also determined. The specific conductivity of the sap expressed from the main-axis leaves and from the bark was measured in the usual way (cf. Mason and Phillis, 1942*a*). At 3.00 p.m. on the evening before collection, the plants were watered and placed in a dark-house where they remained until collected next morning at 8 a.m.

III. RESULTS

The results for the lamina for hydration on both dry weight and protein bases, for conductivity of the expressed sap, and for the dry weight of the whole plant (i.e. size) are plotted against potassium supply in Fig. 1. The size was greater in the 'Dry' than in the 'Wet' series and was also greater at

the second than at the first collection. As the potassium supply increased there were well-marked increases in dry weight. In the 'Dry' series at the
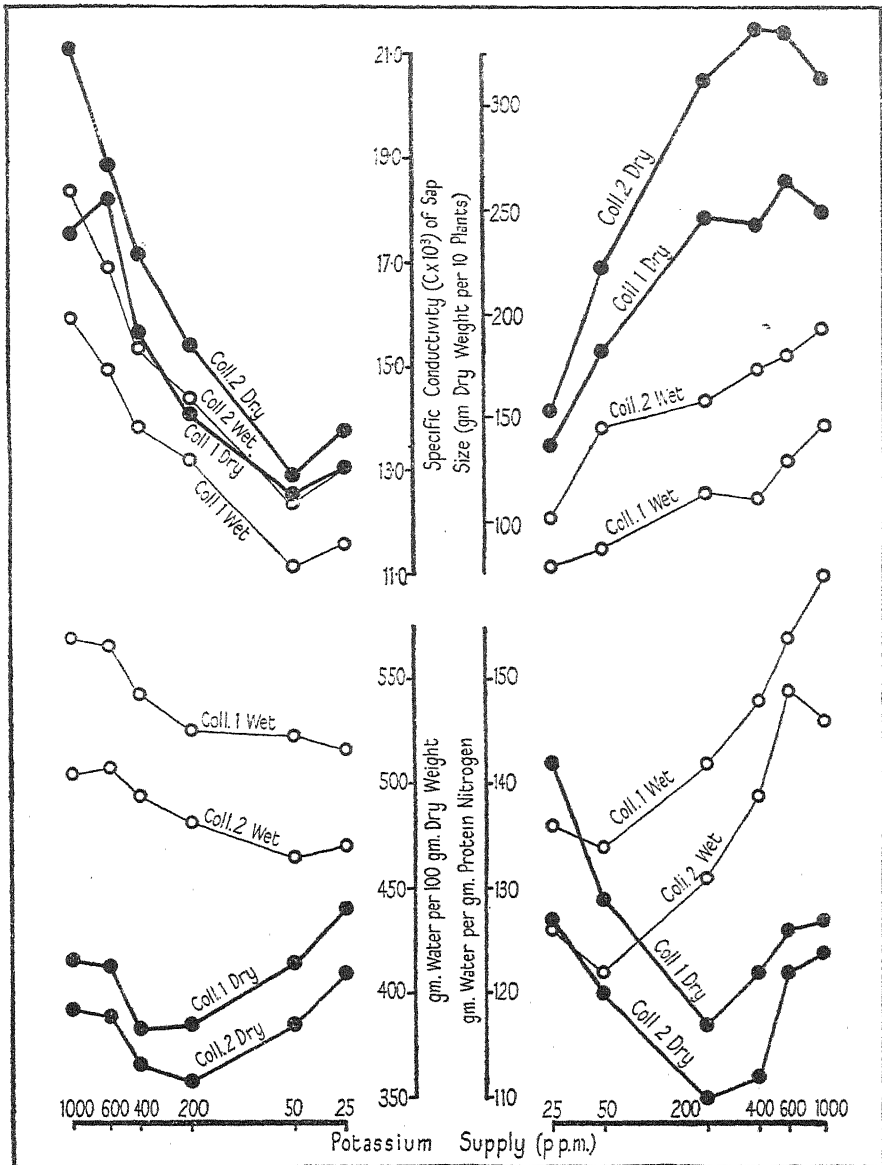

FIG. 1. Size (gm. dry weight per 10 plants), conductivity ($C \times 10^3$) of sap expressed from lamina, and hydration of lamina on dry weight and on protein basis, under 'Wet' and 'Dry' conditions for varying levels of potassium supply.

second collection the size actually diminished between the 600 and 1,000 p.p.m. potassium supply.

The specific conductivity of the expressed sap was also greater in the 'Dry' than in the 'Wet' series and at the second than at the first collection. As the potassium supply was increased there were in both series initial declines in conductivity and then well-marked increases up to the highest level of potassium supply.

Coming to hydration we find, as might be expected, that hydration both on the dry weight and on the protein bases is less in the 'Dry' than in the 'Wet' series. It is also less at the second than at the first collection. The differences between the two series are rather more marked on the dry weight than on the protein basis. The actual changes due to variation in potassium supply are not unlike for the two bases of expression for the 'Dry' series. Initial declines were later followed by smaller increases. In the 'Wet' series the main difference between the results on the two bases is that hydration on the protein but not on the dry weight basis showed an initial drop. *The most important feature, however, is that hydration tended to rise in the 'Wet' series and to fall in the 'Dry' series.*

Inspection of Fig. 1 suggests that in the 'Wet' series the changes in hydration caused by variation in the supply of potassium might be related to changes in sap conductivity. When hydration is expressed on the protein basis the changes in hydration and in conductivity are very similar. It will, however, be noticed that the conductivity is higher at collection 2 than at collection 1, and that the reverse is true for hydration. The changes in hydration in the 'Dry' series, on the other hand, appear to be related to changes in size rather than to changes in conductivity.

In Fig. 2 we have plotted hydration against size (lower figures) and against conductivity (upper figures). On the left of the figure, hydration is expressed on the dry weight basis and on the right on the protein basis. In the 'Wet' series, *hydration,* on both bases, and *conductivity* show fully significant, positive linear correlations. In the 'Dry' series *hydration* and *conductivity* are not (linearly) related. It would appear that while conductivity might have been a factor determining the changes in hydration in the 'Wet' series, other factors were at work in the 'Dry' series and, moreover, were responsible even in the 'Wet' series for the drop in hydration between collections 1 and 2.

The correlations between *size* and *hydration,* on both bases, are positive and fully significant in the 'Wet' series. This may be due to the fact that conductivity and size are strongly correlated. In the 'Dry' series, *hydration* and *size* are not (linearly) related. Hydration diminished, however, as long as size showed well-marked increases. At high levels of potassium supply the changes in size were small and hydration increased, presumably because of the increases in conductivity.

It is rather surprising to find that hydration in the 'Wet' series is so much less at collection 2 than at collection 1. This is true for both the dry weight and the protein basis of expression and may perhaps be due to the increase in resistance to the movement of water within the plant. The mean relative changes in hydration and size for both the 'Wet' and the 'Dry' series between

the two collections are shown in Table 1. It will be noticed that the mean changes in hydration (on the dry weight basis) and in size are greater in the 'Wet' than in the 'Dry' series.
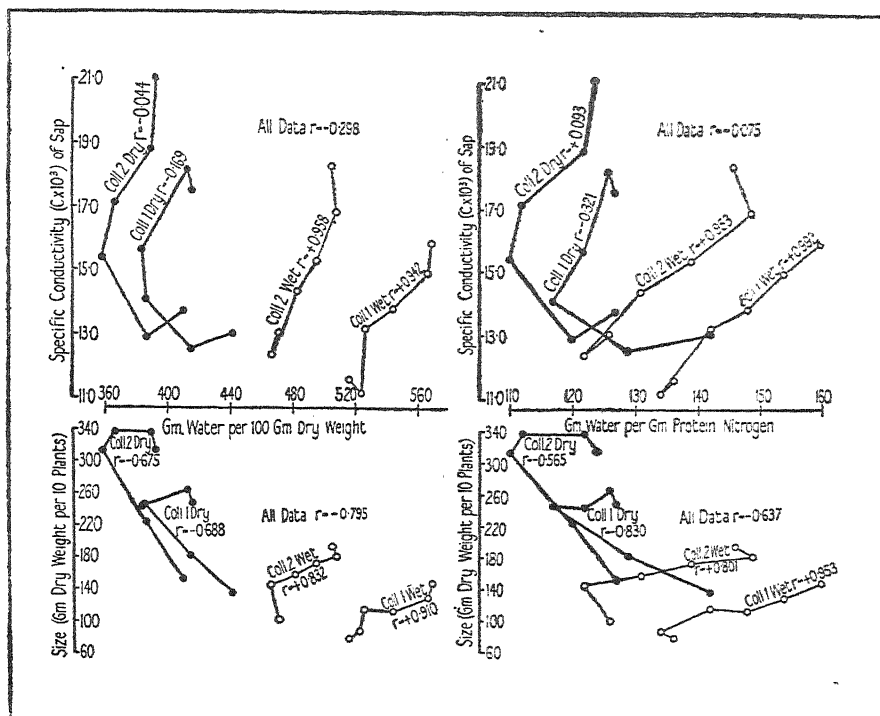

FIG. 2. Hydration of lamina in relation to conductivity and size. Upper figures, conductivity and hydration on dry weight basis (left) and on protein basis (right). Lower figures, size and hydration on dry weight basis (left) and on protein basis (right).

TABLE I

Mean Percentage Changes in Hydration and in Size between Collections 1 and 2.

	Hydration:		Size.
	Dry Wt. Basis.	Protein Basis.	
'Wet' series	−10	−7	+44
'Dry' series	− 6	−6	+25

To sum up, hydration in the 'Wet' series in as far as it is affected by changes in potassium supply seems to be related to changes in conductivity, while in the 'Dry' series the size obtrudes itself as the more important factor. It would also appear that size may be a factor even in the 'Wet' series as the plant ages. The results for the bark were essentially similar to those of the leaf and are not reported.

IV. Discussion

The results suggest that an increase in the supply of potassium leads to an increase in the concentration of electrolytes and that this is accompanied by an increase in hydration. This, however, appears to be true only for humid conditions. When potassium supply leads to well-marked differences in the size of the plant and the environment is arid, the true potassium effect is obscured as a result of differences in water strain, and hydration is negatively correlated with size. This appears to be generally true whether hydration is expressed on the dry weight or on the protein basis, and for both the leaf and the bark.

In the 'Dry' series it has been assumed that the water strain was greater in a large than in a small plant. This of course might occur as a result of a greater leaf area or as a result of a greater length of conducting tracts. If the saturation deficit is really greater in a big than in a small plant then, when transpiration is checked and the plants reach water equilibrium, the differences in water strain should be eliminated and hydration should be independent of the size of the plant. Actually this was not so, for the plants of the 'Dry' series were placed in a dark-house, watered, and left until next morning before sampling, yet hydration and size were negatively correlated. If this were true only for hydration on the dry weight basis then the explanation might be that size of plant and size of cell were inversely correlated, for the smaller the cell the greater will be the amount of wall material per unit volume of tissue. Yapp, in a footnote to his translation of Maximov's (1929) book, has discussed the effects of water strain on developing leaves and has indicated how profoundly the morphological structure of the leaf may be changed by drought. The negative correlation between size and hydration also holds, however, when the latter is expressed on the protein basis and so the cause of this correlation would appear to be independent of morphological changes. It may be that a continuous or fluctuating water strain modifies the hydration capacity of protoplasm, possibly through some effect on structure. The problem is one of considerable interest in connexion with the basis of drought resistance.

V. Summary

1. The plants were grown with varying potassium supply under 'Wet' and 'Dry' conditions. Dry weight of whole plant, hydration in the leaf and in the bark (in terms of dry weight and of protein), and the specific conductivity of leaf and bark sap were determined.

2. It was found under 'Wet' conditions and also under 'Dry' conditions that as long as increases in potassium supply caused little or no further increases in dry weight, increased potassium supply caused increased hydration. This increased hydration was associated with increased sap concentration. Thus the effect of potassium on hydration falls into line with the salt concentration hypothesis of hydration.

3. Size of plant *per se* affects the hydration of the leaves under 'Dry' conditions. It also affects the hydration under 'Wet' conditions as the plants age, possibly as a result of an increase in resistance to the movement of water within the plant.

4. Hydration in the bark undergoes very similar changes to those in the lamina in response to changes in potassium supply.

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Studies on the Partition of the Mineral Elements in the Cotton Plant

IV. More about Nitrogen, Phosphorus, and Labile Carbohydrate[1]

BY

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With four Figures in the Text

I. INTRODUCTION

IN a recent paper (Phillis and Mason, 1942) we showed that in the leaf the partition index for nitrogen (i.e. protein-N expressed as a percentage of total-N) was negatively correlated $(r = -0.994)$ with crystalloid-N per 100 gm. dry weight. It was concluded that the main factor controlling the protein level (i.e. protein-N per 100 gm. dry weight) was the crystalloid level (i.e. crystalloid-N per 100 gm. dry weight). As the crystalloid level increased, the protein level increased until it reached a maximum value, after which further increases in the crystalloid level brought about a reduction in the protein level. These changes, it was pointed out, were characteristic of apolar adsorption. No single experiment, however, covered the whole range of protein increase and decline, and it might be argued that the observed relation between protein and crystalloid-N was due to the choice of the four particular experiments rather than to any common unifying relation. In the present paper a single experiment is described which covers the necessary range.

[1] Paper No. 33 from the Physiological Department of the Cotton Research Station, Trinidad.

Our previous paper also contained some data which indicated that the partition of phosphorus between soluble and insoluble forms might conform to the same pattern as that for nitrogen. These data dealt with variations in phosphorus partition under varying nitrogen supply, where nitrogen was limiting growth and phosphorus was in excess. An experiment which deals with the partition of phosphorus over a wide range of phosphorus supply, ranging from marked insufficiency to excess, is described in the present paper.

In our previous paper we also presented data which suggested that the partition of labile carbohydrate might also conform to an adsorption pattern. Labile carbohydrate contained two fractions, viz. total sugars and polysaccharides. The variation in polysaccharides was, however, very small. In the present paper we have determined starch in place of polysaccharides and have succeeded in bringing about large variations.

II. Variation in Nitrogen (*Experiment 1*)

A. *Procedure.*

Sea Island cotton plants were grown in sand culture under glass. There were eight levels of nitrogen (ammonium nitrate) supply ranging from 50 to 2,500 p.p.m. The plants were collected when 38 days old. On the main-axis leaves, dry weight, total-N, and crystalloid-N were determined. The dry weight of the whole plant was also determined. The sampling and collection followed our usual procedure.

B. *Results.*

The results are shown graphically in Fig. 1. On the left of the figure the dry weight of the whole plant and crystalloid-N and protein-N per 100 gm. dry weight in the leaf are plotted against nitrogen supply. It will be seen that dry weight increased until the fourth level of supply and then declined, and that the dry weight at the highest was much the same as at the lowest level of supply. The cause of this depressing effect of excess supply (of ammonium nitrate) is not known. Crystalloid-N per 100 gm. dry weight increased with increasing supply. Protein-N per 100 gm. dry weight increased to a maximum value at the fifth level of supply and then decreased slightly. *This depression in protein-N per 100 gm. dry weight is fully significant.* The maximum value for protein-N per 100 gm. dry weight did not coincide with maximum dry weight of the whole plant, nor was the depression for protein as marked as for dry weight.

On the right of Fig. 1 (top), protein-N is plotted against crystalloid-N, both expressed per 100 gm. dry weight. Below the partition indices (see introduction) for nitrogen are plotted against crystalloid-N per 100 gm. dry weight. It will be seen that the partition indices are highly correlated with the concentration of crystalloid-N and the points all lie on a straight line. This straight-line relation has enabled us to calculate a theoretical curve for

the relation between protein-N and crystalloid-N. This curve is shown in the top figure. The experimental points fit very closely to the curve. Moreover, they cover both sides of the protein maximum.

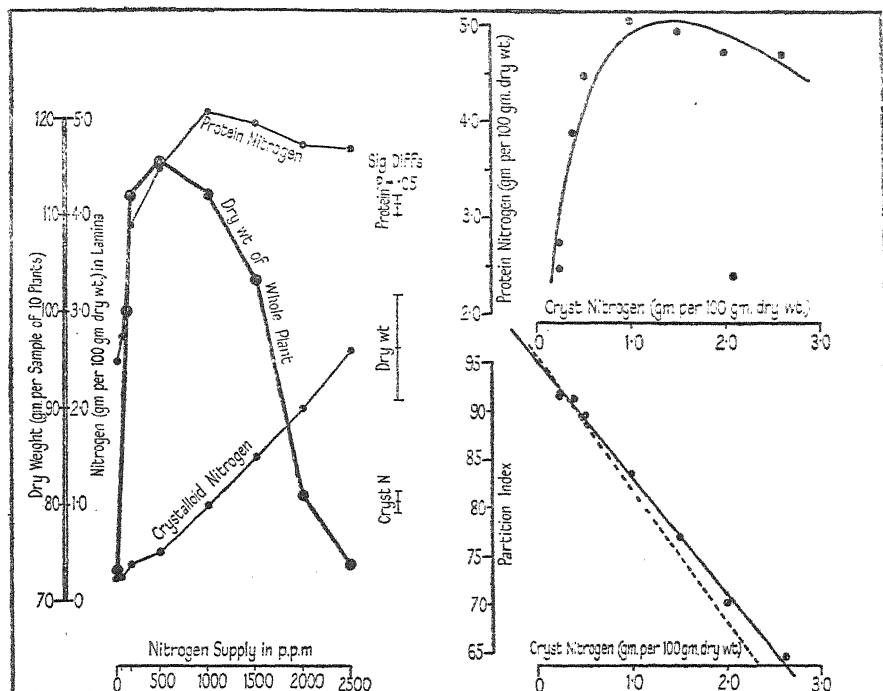In addition to the regression line for the present experiment, we have

FIG. 1. The dry weight of the whole plant (gm. per 10 plants) and weights of protein-N and crystalloid-N (per 100 gm. dry weight) in main-axis leaves plotted against N-supply (left). Partition indices (lower fig.) and protein-N (upper fig.) plotted against crystalloid-N per 100 gm. dry weight (right). The continuous line (lower right) is the calculated regression of the partition index on crystalloid-N concentration, while the broken line is the regression from previously reported experiments. The calculated regression was used in constructing the curve (upper right) relating protein and crystalloid-N concentrations.

included in the bottom figure the regression line between crystalloid-N per 100 gm. dry weight and the partition indices from our previous nitrogen-partition paper. The two lines do not coincide but the divergence is small. In our previous paper we pointed out that the only additional factor we had found to affect partition was age of leaf. Actually the divergence between the two regression lines is of the same order as that found between leaves of different ages on the same plant, and may well be due to the fact that in the present experiment we used younger plants than usual.

To sum up, the results of the present experiment confirm the conclusions reached in our previous paper concerning the relation between crystalloid-N and protein-N. Furthermore, the relation between crystalloid-N and the

partition indices appears to be the same in this experiment, making a slight allowance for age, as was found for the combined data in the previous paper, thus strengthening our conclusions that crystalloid-N level is the main factor determining nitrogen partition in the leaf.

III. Variation in Phosphorus (*Experiment 2*)

A. *Procedure.*

Sea Island cotton plants were again grown in sand culture. Half the plants were grown under glass and the other half under mosquito wire. There were seven levels of phosphorus supply (sodium acid phosphate) ranging from 10 to 600 p.p.m. The supply of other elements was maintained at a constant level (nitrogen 300 p.p.m.; potassium, calcium, and magnesium 100 p.p.m. each). Trace elements were added as usual. The sand around the roots was leached weekly with water before the addition of fresh solution. Sap-soluble and total phosphorus were determined in the leaves from the main axis.

B. *Results.*

The results are given in Fig. 2. On the left are shown the dry weight of the whole plant and the weights of soluble and insoluble phosphorus per 100 gm. dry weight in the leaf for the wire house only, plotted against phosphorus supply. The results for those grown under glass were generally similar and are not shown. It will be seen that the dry weight increased up to the fourth level of supply, while further increases in phosphorus supply decreased the dry weight of the whole plant. In the leaf both soluble and insoluble phosphorus expressed in terms of dry weight increased with increasing supply. Soluble phosphorus changed more than insoluble phosphorus. It began at a lower and finished at a higher level than the insoluble fraction. Insoluble phosphorus continued to increase with increasing supply and is therefore unlike protein which reached a maximum and then declined. It may be that the ratio of soluble to insoluble phosphorus will prove a valuable guide to the phosphorus status of the plant.

On the right of Fig. 2 soluble and insoluble phosphorus *for both the glass and the wire houses* are plotted against each other in the upper figure, and below the partition indices for phosphorus are plotted against the concentration of soluble phosphorus per 100 gm. dry weight. It will be obvious that the difference in environment between the two houses has had no appreciable effect on the partition of phosphorus. The correlation coefficient between the partition indices and soluble phosphorus concentration is remarkably high ($r = -0.986$), but it is doubtful whether the correlation is linear. We have, however, assumed linearity for the purpose of calculating the curve between soluble and insoluble phosphorus. This curve is shown at the top of the figure. The closeness of fit of the experimental points to the calculated line is not as good as it was for nitrogen.

For the purpose of comparison the regression line for partition index on soluble phosphorus given in the previous paper is shown along with the regression line for the present experiment. Unlike the nitrogen lines they are
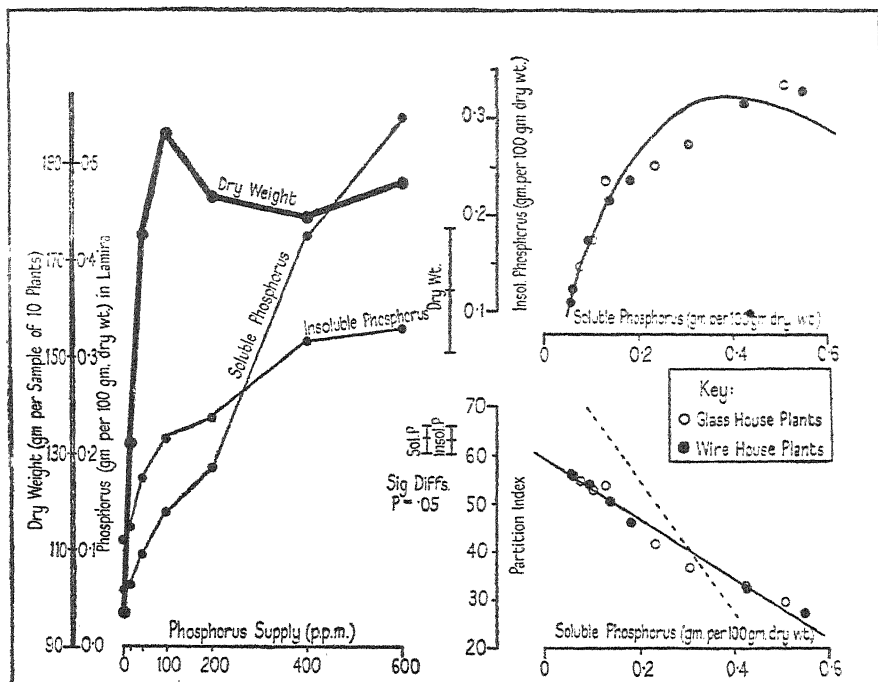

FIG. 2. The dry weight of the whole plant (gm. per 10 plants) and weights of soluble and insoluble phosphorus (per 100 gm. dry weight) in leaves of main axis plotted against P-supply (left). Partition indices (lower fig.) and insoluble phosphorus per 100 gm. dry weight (upper fig.) plotted against soluble phosphorus per 100 gm. dry weight (right). The continuous line (lower right) is the calculated regression of the partition index on soluble phosphorus concentration, while the broken line is the regression from previously reported experiments. The calculated regression was used in constructing the curve (upper right) relating insoluble and soluble phosphorus concentrations.

far from coinciding. We have found that each experiment gives its own particular and distinct line for phosphorus.

Previous data on phosphorus partition were concerned with a state in which phosphorus was in excess and in which soluble and insoluble phosphorus were negatively correlated. These data suggested that phosphorus would behave like nitrogen in that as supply was increased from starvation to excess, insoluble phosphorus would rise to a maximum and then decline. When the present experiment was laid down it was thought that the higher levels of supply would be sufficient to reach beyond the maximum for insoluble phosphorus and that there might be a decrease. This has not occurred, for there is no indication that even the maximum value for insoluble phosphorus was attained. The data do fit in with expectation, however, in that in the region

before the maximum value is attained there is a positive correlation between soluble and insoluble phosphorus. Phosphorus partition resembles that for nitrogen, and it may be that a similar process is at work in the two cases, but it is clear that a single experiment covering both sides of the maximum is required before any definite conclusion can be drawn.

IV. VARIATION IN LABILE CARBOHYDRATE (*Experiment 3*)

A. *Procedure.*

In this experiment the leaf disc technique (Mason and Phillis, 1942) was used. This technique was used as much greater variation in labile carbohydrate can be obtained in this way than when leaves are still attached to the plant. First of all the discs were cut and floated on water in trays. They were exposed to the normal diurnal cycle of light and darkness. On the day after cutting they were divided up as follows: three groups were left floating on water, one for the initial and two for subsequent collections; these constituted the 'Water' treatment. Other discs were floated on 3 per cent. and on 6 per cent. sucrose solutions. Collections were made one and four days after the initial collection. At each collection there were two samples of 250 discs for each treatment.

Time-Table

Day 0. Initial collection.
,,   1.   1st collection of 'Water' and 3 and 6 per cent. sucrose treatments.
,,   4.   2nd collection of 'Water' and 3 and 6 per cent. sucrose treatments.

Total sugars were estimated on the expressed sap after invertase hydrolysis. The method for the estimation of starch followed the procedure recommended by Sullivan (1935), i.e. extraction with calcium chloride, with the exception that the purification of the crude starch precipitate by reprecipitation with iodine was omitted owing to filtration difficulties. The values given for starch are really the actual weights of glucose obtained. In order to allow time for the attainment of equilibrium between starch and sugar, discs to be collected on any particular day were kept darkened from sunrise until the time of collection, which was between 8 and 10 a.m., so that at collection they had been in the dark for a continuous period of about 15 hours.

B. *Results.*

The results on the sample basis are shown in Fig. 3. On the left, starch, and on the right, total sugars are plotted against time. The results represent the weights of starch and sugar respectively in 1,000 discs.

It will be seen that there were considerable increases in both starch and sugar. In the 'Water' treatment both starch and sugar increased at approximately uniform rates throughout the four days of the experiment. Small increases in sugar were accompanied by much larger increases in starch. In

this treatment the increase in starch and sugar during the experiment must have been produced wholly by photosynthesis, for the increase in dry weight exceeded that of the combined increase due to starch and to sugar.
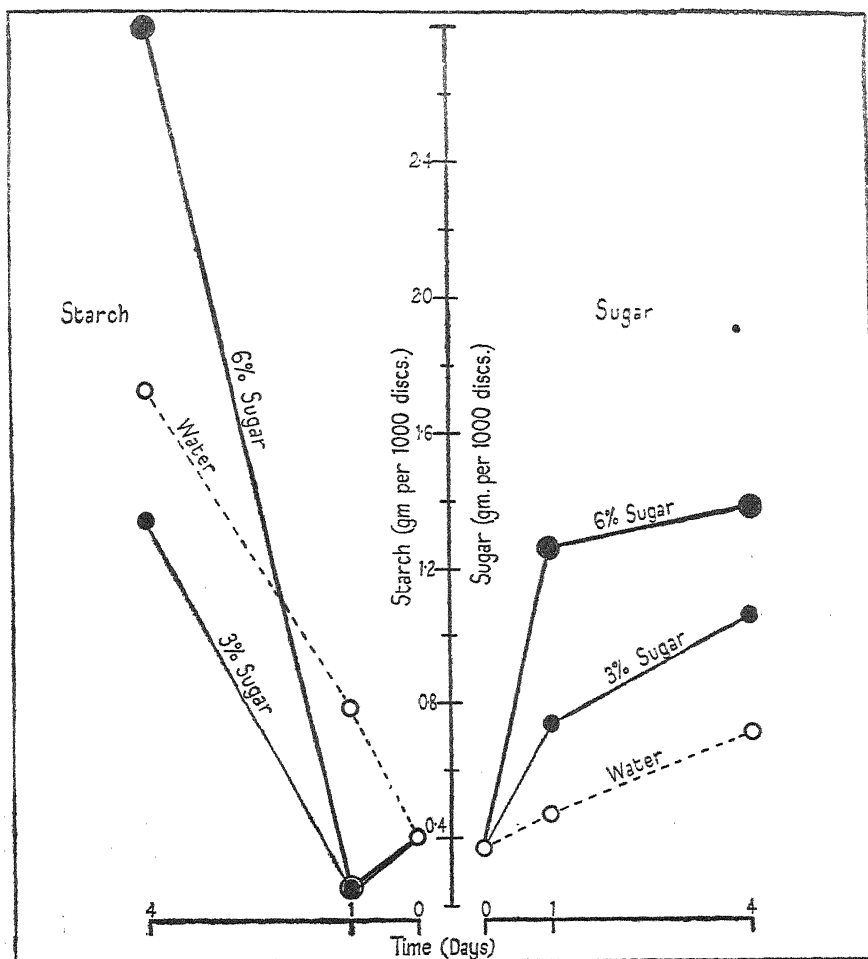

FIG. 3. Curves of starch (left) and sugar (right) in terms of gm. per 1,000 discs for 'Water' and two 'Sugar' treatments plotted against time.

In the discs floating on the sugar solutions there were during the first day increases in sugar and decreases in starch. Moreover, the increases in sugar exceeded the decreases in starch. The cause of the decrease in starch is not clear (cf. Phillis and Mason, 1937). Between the first and second collections there were only small increases in sugar and very large increases in starch. In the two sugar treatments the increases in labile carbohydrate were presumably due both to absorption of sugar and to carbon assimilation. The increases in dry weight again exceeded the increases in labile carbohydrate.

In Fig. 4 sugar and starch are plotted against one another. Both are expressed in terms of dry weight. It should be explained that the graph is not
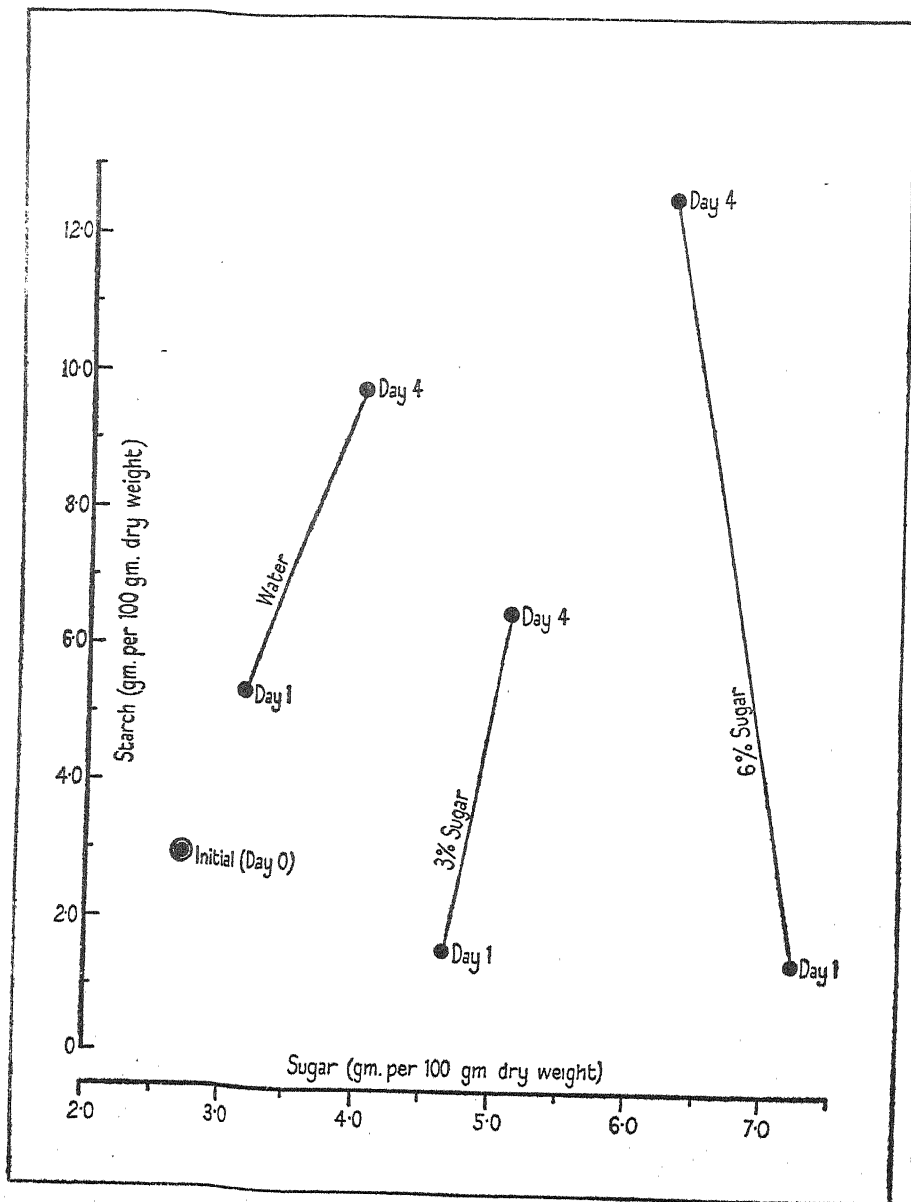

FIG. 4. Starch (gm. per 100 gm. dry weight) plotted against gm. sugar per 100 gm. dry weight) for initial and subsequent collections of 'Water' and two 'Sugar' treatments.

materially changed when the results are expressed per 100 gm. water. The values at collections 1 and 2 for each of the three treatments are joined by lines. It

will be seen that the initial collection and 'Water' treatments show a nearly linear relation between starch and sugar. As the external sugar concentration is increased we find that between collections 1 and 2 starch shows large increases with no appreciable change in sugar per 100 gm. dry weight. The results suggest that, unlike nitrogen and phosphorus, the partition of labile carbohydrate does not follow an adsorption pattern, but rather that the soluble fraction under any given condition tends to approach a limiting value.

V. DISCUSSION

All our investigations on the partition of nitrogen in the leaf between soluble and insoluble fractions have been carried out on plants in a vegetative state. Within this limit the only factor affecting the partition of nitrogen in addition to the crystalloid-N concentration is the age of the leaf, and its importance is relatively small. In short, all our partition data for nitrogen fit closely to a single regression line. This does not, however, appear to be the case for phosphorus, where each experiment appears to have its own distinct regression.

Most of the soluble nitrogen is in organic form, whereas with phosphorus much of the soluble fraction consists of phosphates. Again, the whole of the insoluble nitrogen in the leaf is believed to exist in some form or forms of organic combination, whereas with phosphorus there are both organic and inorganic forms to consider. A considerable proportion of the insoluble phosphorus appears to exist as calcium phosphate. It seems unlikely that such inert material would play any part in an adsorption relation governing the partition, and it should be allowed for in estimating the equilibrium between soluble and insoluble forms. If, when this has been done, there is still variation from experiment to experiment in the partition-concentration relations, it may be possible to use such variation in an attempt to identify the adsorbent which makes possible such equilibrium.

With regard to labile carbohydrate the situation is far from clear. The starch level is evidently dependent on factors other than the concentration of sugar (cf. Ahrns, 1924). In this connexion it must be recollected that starch is localized in the chloroplasts and is not, like protein and part of the insoluble phosphorus, distributed throughout the protoplasm. A further difference is that starch is not a constituent of the protoplasm.

VI. SUMMARY

1. The relation between crystalloid-N and protein-N has been investigated in an experiment in which nitrogen supply was varied from acute starvation to considerable excess. The crystalloid-N concentration (in terms of dry weight) rose continuously with increasing supply, while the protein concentration rose to a maximum value and then declined. The relation between crystalloid-N and protein-N follows an adsorption (apolar) pattern.

2. In a second experiment, phosphorus supply was varied from acute

starvation to excess. The concentrations of both sap-soluble and insoluble phosphorus rose continuously with increasing supply. It had been anticipated from previous experiments that insoluble phosphorus would decline as the phosphorus supply reached high levels, but no such decline was noted in the present experiment. The data of this and of previous experiments considered together are in harmony with the view that the relation between soluble and insoluble phosphorus is of the same type as that between crystalloid-N and protein-N.

3. The relation between crystalloid-N and protein-N remains relatively constant from experiment to experiment in spite of widely divergent conditions, but this does not appear to be so in the case of phosphorus, where the soluble/insoluble phosphorus relation changes markedly from experiment to experiment. It is pointed out that most of the nitrogen in the leaf is present in organic form, while much of the phosphorus is present as phosphates.

4. It is also shown that the relation between starch and sugar is controlled by factors quite unlike those that prevail for nitrogen and phosphorus.

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